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THE BLOOD LIPIDS IN THE POSTABSORPTIVE STATE AND AFTER THE INGESTION OF FAT IN NORMAL HUMAN SUBJECTS AND IN A CASE OF DISSEMINATED CUTANEOUS XANTHOMATA [1]

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The presence of hypercholesterolemia in xanthomatosis attracted the attention of early workers, but it was soon shown that an increased blood

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ent the different lipids present.

The present study was undertaken with a view to obtaining more information concerning the variations in the blood lipids in xanthomatosis under various physiological conditions. It seemed to us that such an experimental approach might throw light on the nature of the lipid disturbance in xanthomatosis. The metabolism of a patient presenting cutaneous xanthomata was therefore investigated in the following respects: (1) the levels of the blood lipids in the postabsorptive state during a prolonged period of observation; (2) the effect of a short fast upon the blood lipids; (3) the quantitative relation of total fatty acids, free cholesterol and ester cholesterol of the blood; (4) the response of the blood

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lipids to ingested fat.   A somewhat similar study was made on several normal subjects to serve as a basis for comparison.

EXPERIMENTAL

Twelve young adults, 10 males and 2 females, varying in age from 17 to 39 years, served as subjects for the study of the normal fat metabolism. No attempt was made to regulate the previous diet or nutritional state of the subjects other than to withhold all food for 10 to 14 hours before the beginning of the experiment.   After a sample of blood had been taken for the determination of the fasting lipid values, 7 of the subjects received 100 cc. of olive oil flavored with a few drops of oil of spearmint.   They were also permitted to drink 200 cc. of tap water immediately after the ingestion of the oil.   Blood was taken as a rule at 2-hour intervals over a period of 10 hours.   During the period of observation the subjects either slept or rested.

The history of the patient who suffered from cutaneous xanthomata follows:

E. W., an unmarried, white American male, a carpenter, 27 years of age, was admitted to the medical clinic on September 19, 1931, complaining of a papular and nodular eruption on both knees, which had begun 2 years prior to the date of his entry and had at first manifested itself by a slight pain on flexion of the knee.   Within 6 months after the appearance of the tumors in the region of the knee similar lesions became visible on the fingers and palmar surfaces of both hands and about the extensor surfaces of the elbows.   During the 6 months prior to his admission nodules also appeared over the buttocks and along the entire posterior surface of both thighs.   These tumor-like masses were firm, yellow or saffron colored, and varied in diameter from 2 mm. to 1.5 cm.   The lesions were painless, although discomfort was experienced upon pressure.   It is worthy of note that the patient had an aversion to fats, milk, and eggs.   His weight on admission was 72 kilos and his height 162.5 cm., indicating a well developed male.   Past illnesses included measles and whooping cough in childhood, appendicitis at the age of 17, and a Neisserian infection of 6 to 7 weeks' duration at the age of 18.   The family history was negative.   At the time of admission the urine was normal and the fasting blood sugar was 110 mgm. per cent.   The glucose tolerance test was normal.   As judged by the rose-bengal test, there was no liver dysfunction and cholecystography showed a normally functioning gallbladder.   The basal metabolic rate was minus 6 per cent.   X-ray examination revealed no bony changes in skull, chest, feet, or legs.

The treatment of E. W. previous to and during the ingestion of 100 cc. of olive oil was similar to that recorded above in the case of the normal subjects. The procedure in the fasting experiment was similar to that of the other experiments, except that no oil was ingested.

With 2 exceptions, the ingestion of oil was without ill effects upon normal subjects and patients.   W. H. and E. W. (the latter during his first experiment only) were slightly nauseated for a short time following the administration of the oil.

A single fat rather than a diet high in fat was used, inasmuch as this procedure presented less danger of variation in the fat content of the test meal from time to time.

Sampling and storage of blood. Venous blood taken from the forearm was oxalated and pipetted with continuous stirring into a flask containing 25 cc. of redistilled 95 per cent alcohol. Ten cc. of blood were taken from normals and 5 cc. from the patient with xanthomata. The flasks were stoppered with tinfoil-covered corks and stored in the dark at minus 1° C. until analyzed.

Extraction of blood. The blood was extracted by a modification of Bloor's method (3). Peroxide-free, redistilled ethyl ether was added until the volume of solvent reached 75 cc. The mixture was refluxed in a water bath for one hour at 55° ± 5° C., with vigorous rotation of the contents at intervals. An all-glass coil, which at the same time served as a cover to the flask, was used as a condenser. After cooling, the entire mixture was transferred quantitatively to a 100 cc. glass-stoppered volumetric flask. The contents were made up to volume at 20° C. and then filtered through fat-free filter paper into glass-stoppered flasks. While samples were removed for analyses, the extract was kept at 20° C. All determinations were done in duplicate, and the figures reported are averages of values which checked within 5 per cent.

Determination of cholesterol (free and total). Free cholesterol and total cholesterol were determined after the manner of Okey (4), with a few modifications suggested to us by Dr. Okey herself. Carbon dioxide was used instead of air as the agent for removing the last traces of solvent. In the first analysis on E. W. a saturated solution of potassium hydroxide was used as the saponifying agent instead of sodium hydroxide. Sodium ethylate, freshly prepared according to Bloor (5), was substituted in all other work. The time of oxidation was extended to 40 minutes.

Determination of total fatty acids. Total fatty acids were determined by the method of Bloor (5).

Determination of total lipid. This was calculated as the sum of total fatty acids and total cholesterol.

Okey and Stewart (6) point out that slight irregularities in the effects produced by anticoagulants and by centrifugation make plasma less desirable than whole blood for comparative lipid studies. Moreover, it has been shown that the corpuscles participate in lipid transport, for the administration of fat to dogs invariably results in an increase of the fatty acid content of the corpuscles as well as of the plasma. In this investigation, therefore, the use of whole blood for comparative lipid determination was adopted.

RESULTS

Normal subjects

The blood lipid studies in normal subjects are summarized in Tables I and II.

1. In the postabsorptive state. The total lipid content of whole blood varied from 448 mgm. to 610 mgm. per 100 cc., while the minimum and maximum values for fatty acids were respectively 310 and 432. These figures are in close agreement with those obtained in recent years by the oxidative procedures (7, 8, 9). Okey and Boyden (10) have made the interesting observation, later confirmed by Kaufmann and Mühlbock (11) and by Okey and Stewart (6), that a definite fall in the blood cholesterol of women occurs during or near to the menstrual period, and in a few cases a similar tendency for cyclic changes in the fatty acids of the blood

TABLE I

Constituents of whole blood lipids in normal fasting subjects

Subject	Sex	Total lipid	Total fatty acids	Cholesterol		
				Free	Ester	
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent of total*
B. S.	Male	594	400	122	72	37.1
C. E. H.	Male	600	432	101	67	39.9
		610§	424	111	75	40.3
W. H.	Male	488	310	98	80	45.0
A. K.	Male	561	370	112	79	41.4
I. L. C.	Male	459	316	95	48	33.6
T. H. M.	Male	607	412	102	93	47.6
A. Ki.*	Female	518	365	95	58	37.9
B. L.	Male	477	333			
R. C.†	Male	563	425			
E. M.‡	Female	448	331			
H. L.	Male					
Maximum		610	432	122	93	47.6
Minimum		448	310	95	48	33.6
Average		539	374	105	72	40.4

* 18 days after cessation of menstruation.
† Blood clotted very rapidly.
‡ 17 days after cessation of menstruation.
§ Samples taken 5 months apart.

was also noted. Hence blood-lipid figures in women can be of comparative value only if obtained during the intermenstrual period. Such observations in the plasma lipids of 8 young women have recently been reported by Boyd (12).

The total cholesterol values obtained in 12 normal subjects were 117 mgm. per 100 cc. of whole blood for the lowest and 195 for the highest; the free or uncombined portion of this consisted of 95 mgm. in the case of the minimum value and 122 mgm. in the case of the maximum, whereas the esterified portion varied from 48 to 93 mgm. The average figures for total, free, and ester cholesterol were 166, 105, and

72 respectively. The latter agree closely with the mean values reported by Okey and Stewart (6).

Total fatty acids were present to the extent of 70 per cent of the total whole blood lipids in the normal subjects examined. In plasma, Boyd (12) found that fatty acids constituted 60 per cent of the total lipids, whereas McQuarrie, Bloor, et al. (9) found 65 per cent as fatty acids. Total cholesterol, which amounted to 30 per cent of the total whole blood lipids, had in our normal subjects an average ester content of 40 per cent. This proportion of ester to total cholesterol corresponds closely with the mean values in whole blood, namely, 39, 42, and 42 per cent, reported for 3 different diets by Okey and Stewart (6), but is lower than that obtained for plasma by Boyd (12).

2. *The influence of fat ingestion.* The influence of a single meal of fat upon the concentration of lipids in the blood of normal subjects is shown in Table II. The greatest change was demonstrated in the fatty acid component, but considerable non-uniformity was observed in the behavior of the latter in different individuals. Thus, the greatest increase in the total fatty acids was obtained in B. L., in whom this lipid rose 35 per cent above the fasting value at the end of 2 hours, while lesser increases varying from 11 to 27 per cent were observed in B. S., C. E. H., W. H., H. L., and E. M. The time of onset of the rise of the fatty acids and its duration varied in the different subjects examined. In a single case (R. C.) no rise in the level of the fatty acids was observed. The concentration of the fatty acids in the latter subject, however, did not remain constant for at the third and eighth hours decreases were obtained.

With a single exception, no significant change in the cholesterol content of the blood, either free or combined, was produced after a fat meal consisting of 100 cc. of olive oil. In the exception already noted (E. M.), the total cholesterol content of the blood rose slowly and progressively, reaching a maximum value in the fourth hour and showing but a slight decrease from the latter at the end of the period of observation.

Xanthomatosis

1. *In the postabsorptive state.* The fasting level of the lipids in the subject suffering from cutaneous xanthomata is shown in Table III. This patient was observed over a period of 14 weeks, during which time estimations of the fasting blood lipids were made on 5 different occasions. From January 20th to April 28th a rise in the fasting level of all the lipid components of the blood took place. The fasting lipid content of the blood on January 20th was 1160 mgm. per 100 cc., but by April 28th it had risen to the enormous figure of 2180 mgm. per 100 cc. of whole blood, an increase of 88 per cent above the first value. On May 1st the total lipids had dropped to 1920 mgm., a decrease of 260 mgm. per cent in 3 days. As one would expect, the major portion of the total lipids consisted of fatty

TABLE II

The influence of the ingestion of 100 cc. olive oil on the lipids of whole blood in normal human subjects

Subject	Sex	Weight	Oil in-gested per kilo body weight	Hours after fat inges-tion	Total fatty acids	Cholesterol		Total lipids
						Total	Free	
		kgm.	*grams*	*hours*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
B. S.	Male	77.1	1.2	0	400	194	122	594
				2	409	198	122	607
				4	364	188	120	552
				6	422	170	121	592
				8	486	182	120	668
				10	390	190	124	580
C. E. H.	Male	78.9	1.2	0	432	168	101	600
				2	479	172	100	651
				4	340	172	101	512
				6	373	170	101	543
				8	361	163	103	524
				10	356	168	99	524
W. H.	Male	73.9	1.2	0	310	178	98	488
				2	392	181	108	573
				4	369	180	102	549
				6	339	186	106	525
				8	360	184		544
				10	360	156	106	516
H. L.	Male	69.4	1.3	0	Lost	186		
				2	434	186		620
				4	424	176		600
				6	435	182		617
				8	519	187		706
				10	476	190		666
B. L.	Male	68.0	1.4	0	333	144		477
				2	449	146		595
				4	340	143		483
				6	397	149		546
				8	372	150		522
				10	396	151		547
R. C.	Male	72.6	1.3	0	425	138		563
				3	339	144		483
				6	414	141		555
				8	367	146		513
				10	401	144		545
E. M.*	Fe-male	64.9	1.4	0	331	117		448
				2	377	138		515
				4	416	158		574
				6	412	158		570
				8	355	158		513
				10	324	154		478

* 17 days after cessation of menstruation.

acids, and during the marked rise and sudden fall in the total lipids, the fatty acid portion of the latter varied from 72 to 79 per cent, a small but definite increase in this percentage being observed as the total lipid concentration of the blood rose. The percentage increase in total cholesterol was not as great as that which had occurred in fatty acids, for on April 28th, when the fatty acids had already risen by 104 per cent above the value of January 20th, cholesterol had risen by 46 per cent. Throughout the experiment the ester varied from 43 to 53 per cent of the total cholesterol. In the fluctuations of total cholesterol that occurred during the 14 weeks of observation, both free and esterified cholesterol participated, although the percentage increase of each was not the same on different days. On February 5th, a marked increase in ester was found as com-

TABLE III

Components of whole blood lipids in fasting xanthomatosis (E. W.) (Observations over a period of 14.5 weeks)

Date	Total lipids	Total fatty acids	Cholesterol		
			Free	Ester	
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent of total*
January 20...............	1160	840	185	137	42.6
February 5...............	1500	1090	190	216	53.2
February 26..............	1650	1210			
April 28.................	2180	1710	229	241	51.3
May 1...................	1920	1520	198	204	50.8

pared with the value obtained on January 20th, whereas the free cholesterol was practically the same on both days. On April 28th and May 1st, when the total cholesterol rose and fell respectively, free and ester cholesterol shared in these changes to almost an equal degree.

2. The influence of fasting and of fat ingestion. In Table IV are shown the effects of a 10-hour fast and of the ingestion of 100 cc. of olive oil upon the blood lipids in the case of xanthomatosis. Throughout the period of fasting, a drop in the level of the blood fatty acids was found. As compared with the initial value, the fatty acid concentration of the blood showed a decrease varying from 10 to 19 per cent from the fourteenth to the twenty-second hour of fasting. The cholesterol values remained fairly constant throughout this period. Four experiments were carried out with olive-oil feeding. Although the results were not similar in all details, they show, when compared with the fasting control, that the ingestion of oil did not markedly affect the content of the blood lipids in the patient in question. The cholesterol level remained steady during the periods of observation. In one case, the level of the blood fatty acids was below the fasting value throughout the 10-hour interval. In the experi-

ment of April 28th, after a decrease had occurred at the third hour following the meal, there was a slow rise in the concentration of fatty acids till the 8.5-hour interval, at which time the latter had risen by 12 per cent above the fasting level. Following the ingestion of fat on February 5th,

TABLE IV

The influence of fat on the whole blood lipids of a patient with cutaneous xanthomata
(Patient, E. W.; Sex, male; Age, 29)

Date	Hours after fat ingestion	Total fatty acids	Cholesterol		Total lipids
			Total	Free	
1933	*hours*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
February 26	* No fat ingested	1210	436		1650
		1090	432		1520
		980	434		1410
		1040	440		1480
		1080	458		1540
		1040	440		1480
January 20	0	840	322	185	1160
	1½	748	348	184	1100
	3	810	320	188	1130
	5	764	334	188	1100
	7	894	336	182	1230
February 5	0	1090	406	190	1500
	2	944	402	190	1350
	4	944	428	195	1370
	6	1140	404	189	1540
	8	1050	384	188	1430
	10	1060		188	
April 28	0	1710	470	229	2180
	3	1500	446	227	1950
	5	1790	456	220	2250
	6½	1830	476	231	2310
	8½	1920	460	231	2380
	10	1830	478	238	2310
May 1	0	1520	402	198	1920
	2	1420	398	202	1820
	4	1200	404	205	1600
	6½	1430	398	197	1830
	8	1140	415	198	1560
	10	1240	408	208	1650

* Samples taken at 2-hour intervals, starting 12 hours after last meal.

the fatty acids of the blood dropped 13 per cent in the second and fourth hours, and at the other intervals the values fluctuated between a decrease of 4 per cent and an increase of 5 per cent. On January 20th an increase of 6 per cent above the initial value occurred at the seventh hour.

DISCUSSION

If we take the results as a whole, the conclusion seems warranted that no typical or uniform response in the blood fatty acids is produced in normal man by the ingestion of a single fat. When olive oil up to 1.4 gram per kilo of body weight is fed, the effect upon the fatty acids of the blood during the course of the experiment may vary from no rise whatsoever to one involving an increase of 35 per cent. The absence of a typical blood lipid curve may be ascribed in part to the fact that the previous diet of these subjects had not been controlled, or in part to the fact that an interval of 14 hours from the last meal was insufficient to establish a constant nutritional state in the normal subjects. There are numerous other factors, however, which may influence the behavior of the blood lipids towards ingested fat, for this depends not only on the rate of intestinal absorption of the fat, which again is probably dependent upon many factors, but also on the rate at which the tissues remove the absorbed fat from the blood. It is the intestinal factors—as yet poorly understood—that account in part, no doubt, for the normal variability in the response of the blood lipids during the absorption of fat. Rony and Ching (13) believe that under standardized conditions alimentary lipemia curves may serve as an index of the rate of utilization of fat by the organism, but it should be emphasized in this connection that our uncertainty concerning the rate of intestinal absorption entails a fundamental weakness in such an interpretation. It is also important to point out that owing to the limitations of the methods at present employed by various investigators, the blood lipid curves can be of little, if any, value as a routine procedure for detecting alterations in the fat metabolism in pathological conditions.

An attempt to compare the foregoing results with those recorded by others presented difficulties due not only to the profusion of methods used in the estimation of blood lipids but also to the variety of the test meals, which have differed both in kind and in quantity of fat. The older nephelometric methods have been widely employed (14, 15, 16, 17). The use of nephelometric procedures in comparative studies of the blood fatty acids during the introduction of fat absorbed from the intestine has been criticized by Bloor (5), who points out that a sudden change in the composition of the lipids might readily alter their nephelometric properties. Recently, Man and Gildea (18) have employed a titrimetric method in their investigations of the influence of a fat meal upon serum fatty acids. As regards the present investigation, however, the results obtained with the more recently developed oxidative procedures, in which whole blood or plasma has been used, are of more significance. Variations in the response of the blood fatty acids to ingested fat have also been reported by investigators who have used these methods (7, 8, 19).

It seems paradoxical that the level of the blood fatty acids should drop below the fasting value during a 10-hour interval following the ingestion

of fat. Such an effect, however, was obtained in 2 normal subjects (C. E. H. and R. C.). In this respect, these results confirm similar observations made by Hiller et al. (14) and Page, Pasternack, and Burt (7).

The investigations dealing with the effect of a single feeding of fat upon the level of the blood cholesterol have yielded conflicting results. In 6 experiments of the present study no increase in the blood cholesterol was observed following the ingestion of olive oil, whereas in a single case a definite rise of a prolonged nature was found. That a single meal of fat is incapable of influencing to any appreciable extent the level of cholesterol has been reported previously by a number of workers (20, 14, 21, 22, 23). Page et al. (7) were led to the conclusion that the ingestion of 100 cc. of olive oil was followed by an increase in the blood cholesterol, but it should be noted in connection with the results of these investigators that, with the exception of a single case in which a rise of 35 per cent was recorded, the variations observed may be ascribed to diurnal fluctuations during the fasting state—a matter that has recently been investigated by Bruger and Somach (21).

In the present case of xanthomatosis the increase in the blood lipids was reflected in the fatty acid portion as well as in the cholesterol. The fatty acids, moreover, were responsible for the spectacular rise in the total lipids. Although a hypercholesterolemia has been shown by Bloor (24) and others to be an invariable accompaniment of pathological lipemias, the percentage increase in the cholesterol content of the blood is, as a rule, not as great as that in the fatty acids. This same relationship in the percentile increase in the fatty acids and cholesterol was found to hold in the fluctuations that occurred in the degree of lipemia in the present case of xanthomatosis.

Bloor (25) and others have called attention to the constant relationship between the different constituents of the blood lipids. In the 11 normal subjects examined by us in the postabsorptive state, cholesterol made up from 25 to 37 per cent of the total lipids, whereas, in the case of xanthomatosis, cholesterol was present in amounts varying from 21 to 28 per cent. A normal or low proportion of cholesterol in xanthomatosis has also been previously reported (1, 2, 26, 27). With regard to the cholesterol components, it is significant that a greater amount of the cholesterol was in the ester form in our case of xanthomatosis (Table III) than was found in normal subjects.

The fact that on repeated attempts the ingestion of a single meal of fat failed to raise materially the fatty acid or cholesterol content of the blood seems to rule out the possibility that there is any disturbance in the rate at which exogenous fat is removed by the tissues during its transport through the blood. Indeed, the results obtained in this experiment support the view that the removal of absorbed lipids of the tissues is as great as, or greater than, normal. Similar experiments by other observers have also failed to show that in xanthomatosis there is any impairment of the

capacity of the tissues to take up absorbed lipids (26, 27). From the foregoing experiments it appears to be a reasonable inference that the extra load of lipids in the blood in xanthomatosis is not derived from exogenous fat, but has as its source the fat that has been previously stored in the depots or has been synthesized *de novo* from non-fat precursors. The presence of increased amounts of cholesterol as well as fatty acids lends further support to this view, for it has been repeatedly shown that the ingestion of fat, although it may increase the fatty acid content of the blood, is without striking effect upon the blood cholesterol (Table IV; Bloor (22)).

Among the factors that determine the level of the blood lipids in xanthomatosis, the caloric intake may be of importance. After decreasing the diet from 3310 to 2510 calories, Curtis, Wile, and Eckstein (28) observed a fall in the total lipids of the blood in a diabetic patient suffering from xanthomatous tumors. It was not the ingested fat, however, that led to the changed lipid content of the blood, for the decrease in the calories was brought about by the elimination of the major portion of the carbohydrate from the diet, whereas the fat and protein content remained at the previous level. After reduction of the intake below the basal requirement in otherwise normal patients suffering from cutaneous xanthomata, these workers further found a reduction in the level of the blood lipids—which nevertheless was still more than twice the normal value—and simultaneously an involution of the lesions. Apparently the excess fat of blood and tumors is readily available for energy purposes.

SUMMARY

1. Whole blood lipids were determined by means of oxidative procedures in 12 normal subjects in the postabsorptive state.

2. The influence of the ingestion of 100 cc. of olive oil upon the blood lipids in normal subjects was determined. Marked variations in the response of the fatty acids in different individuals were observed. The maximum increase in the fatty acid content of the blood during a 10-hour period of observation was 35 per cent. In 6 out of the 7 normal subjects so studied the ingestion of fat had no appreciable effect upon the cholesterol level of the blood.

3. The limitations in the use of the curve of alimentary lipemia as an index of altered fat metabolism are discussed.

4. The level of the blood lipids in a patient with cutaneous xanthomata was followed for 14 weeks. During this period the total lipid values fluctuated from a minimum of 1160 mgm. to a maximum of 2180 mgm. per 100 cc. The main constituent affected in this rise was the fatty acid portion, which throughout the period of observation constituted from 72 to 79 per cent of the total lipids. The total cholesterol portion varied from 322 to 470 mgm. per 100 cc. of whole blood and composed from 21 to 28

STUDIES ON THE RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND NITROGEN METABOLISM. III. IN POLYCYTHEMIA VERA

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In previous communications we have reported data obtained by simultaneous studies of the nitrogen metabolism and oxygen consumption in cases of pernicious anemia (1) and leukemia (2). We now wish to present similar data obtained by a study of two patients with polycythemia vera who were given phenylhydrazine.

Increased oxygen consumption has been observed in polycythemia vera by many investigators, and numerous explanations for this increased metabolism have been suggested. The subject has been briefly reviewed by Bliss (3) who summarized the explanations given by various investigators to account for the increased metabolic rate frequently observed in polycythemia vera; increased rate of cell regeneration, Abbott (4); increased rate of destruction of nuclear material, Isaacs (5); abnormal blood formation and the presence of immature cells in the circulation, Minot and Buckman (6); and deranged protein metabolism or abnormal physiologic activity of the spleen and bone marrow, suggested by, but not subscribed to, by Grafe (7). Bliss came to the conclusion that the cause of the increased heat production in polycythemia vera was unknown.

The effect of phenylhydrazine upon the rate of oxygen consumption has been touched upon by some investigators. In 1913 Eberstadt (8) gave phenylhydrazine to rabbits and observed a decrease in the oxygen consumption which he attributed to hypoplasia of the bone marrow. The work of Eberstadt was criticized by Rolly (9) who, in 1914, found that phenylhydrazine anemia in the dog was associated with an increase in oxygen consumption. For metabolic work the rabbit is not as satisfactory as the dog since it is difficult, if not impossible, to train rabbits to relax. However, neither of the above investigators give evidence that their animals were trained and only one or two metabolic tests were made during the control periods. Eberstadt's rabbits lost from one-fourth to one-third of their body weight during the experiments, a circumstance which renders most, if not all, of his conclusions invalid. Neither Eberstadt nor Rolly made daily metabolic studies, the former having made two or three deter-

minations per month while Rolly made from six to ten observations per month.

Huffman (10) studied the effect of phenylhydrazine in patients with polycythemia vera and observed an increase in the excretion of nitrogen largely as urea. This author further states that, " Characteristic changes in the heat exchange as indicated by the basal metabolic rate, were not observed." In Huffman's Case 1 the basal metabolic rate increased from 13 per cent above normal to 29 per cent above normal, while the negative nitrogen balance increased from 1.54 gram daily to 9.24 grams daily. In the other cases the basal metabolic rate determinations were made at infrequent intervals and exact dates are not supplied, so that it is not possible to evaluate the results. It is only fair to state that studies on gaseous metabolism seem to have been more or less incidental in Huffman's investigation. Basal metabolic rates before and after treatment with phenylhydrazine, such as are recorded by Bliss, are of no special value to us, since the dates of the determinations are not given and the nitrogen metabolism was not studied simultaneously. Minot and Buckman speak of a case in which the basal metabolic rate was 40 per cent above normal at a time when the erythrocyte count was well below normal. We know of no systematic attempt to point out a relationship between the erythrocyte count and the oxygen consumption, although such relationships have been suggested in leukemia.

We have previously demonstrated that rapid regeneration of erythrocytes in pernicious anemia is associated with a decrease in total oxygen consumption (1). It would therefore seem unlikely that increased regeneration of erythrocytes in polycythemia could cause the opposite effect, as has been suggested by Abbott. The catabolism of nuclear material must require oxygen and this requirement might be enough to produce significant changes in the total oxygen consumption if such material exerts a large specific dynamic effect. However, Ringer and Rapport (11) were able to demonstrate that the metabolism of the dog remained at the basal level for six hours after the ingestion of 20 grams of either yeast or thymus nucleic acid. If it may be inferred from this observation that nucleoprotein can be catabolized by an amount of oxygen similar to, or less than, that needed for like amounts of ordinary protein, then we must conclude that Isaacs' explanation, although valid, is inadequate. Of course, it might be contended that nucleic acid could increase metabolism after an interval greater than six hours, or that it acts differently when, and if, it is liberated outside the gastro-enteric tract. The added suggestion of Minot and Buckman that immature cells in the circulation might use an excess of oxygen has been rendered invalid in the case of leukocytes (12) (13) and inadequate in the case of reticulocytes (2) by respiration studies on blood. Deranged protein metabolism, mentioned, but not subscribed to, by Grafe as a cause

of the increased metabolism in polycythemia, may, in our opinion, be more important than its author thought.

METHODS AND RESULTS

Two patients with typical polycythemia were placed in a metabolism ward. Studies consisted of determinations of nitrogen balance, phosphorus balance, iron balance (14), basal metabolic rate (Tissot-Haldane), blood cell volume (15), hemoglobin (16) erythrocytes and leukocytes as well as observations on the differential count and reticulocyte counts. Observations which were made less frequently included determination of iron (14), phosphorus (17), bilirubin (18) and nonprotein nitrogen in the blood. The viscosity of the blood was determined by the Hess viscosimeter (19). The urine was examined for hemoglobin (20) and for urobilin (21). The amount of hemoglobin as determined by the Newcomer method was compared with the hemoglobin as calculated from the blood iron determinations. The daily intake of iron and phosphorus was calculated from Rose's tables (26). In Case 1 only part of the diet was eaten each day. Two diets were used and were alternated regularly in Case 2. Diet Number 1 contained 12.6 mgm. of iron by calculation and 13.5 mgm. by analysis. Diet Number 2 contained 12.3 mgm. by calculation and 11.2 mgm. by analysis.

The urine from Case 1 which contained hemoglobin was treated with trichloroacetic acid and iron determinations were made separately on precipitate and filtrate. The results of these determinations are shown in Table III. Determinations of albumin, globulin, cholesterol, calcium and phosphorus and phosphorus partition were made on serum or plasma, or whole blood in Case 2, by Dr. G. Stearns of the Department of Pediatrics.

Case 1. A white male, aged 65 years, had had a ruddy face and had suffered from dizziness, weakness and abdominal distress at intervals for four or five years. In August 1930 he developed a partial left hemiplegia and in March 1931 the paralysis of the left side became complete. Thrombosis of the left femoral artery necessitated amputation of the left leg in March 1932, following which the patient died.

Metabolic studies extended from October 19, 1931, to December 15, 1931. The patient's spleen was considerably enlarged and firm. Slight emphysema was the only important abnormality discovered in the thorax. From November 10 until the end of the metabolic period the patient required sedatives at night because of pain, principally in the left foot. On November 16, 1931, determinations of the basal metabolic rate were discontinued because it became necessary to administer morphine at frequent intervals. We feel that the determinations of the basal metabolic rate were unaffected by sedatives except on the last day or two. Some incontinence of urine was observed on the following days: 12, 21, 22, 23, 24, 25, 26, 27, 28 and 32. The amount of urine lost was not at all great except on days 23, 25, and 26, when small amounts were lost on three or four occasions each day. There was no loss of urine in the last three periods when the urine nitrogen was very low.

The amount of phenylhydrazine hydrochloride administered was considerably greater than that which is generally used.

Case 2. A white male, aged 47 years, had had a ruddy complexion for 7 or 8 years. He had a condition which was diagnosed and successfully treated as peptic ulcer in 1924. In 1929 he developed osteomyelitis of the right tarsal bones. Normal function of the foot had not been completely restored at the time of our studies but there were no local signs of inflammation. He complained of distress in the splenic region.

Metabolic studies extended from November 23, 1932, to December 30, 1932. The patient's spleen was only slightly enlarged. There was no demonstrable pulmonary disease. The heart was of normal size by roentgenogram, but there was a systolic thrill and a murmur over the aortic area and an electrocardiogram

TABLE I

Data in Case 1

Day	Hemo-globin, New-comer method	Hemo-globin, iron method	Erythro-cytes	Reticu-locytes	Leuko-cytes	Blood urea nitro-gen	Blood uric acid nitro-gen	Blood bili-rubin	Hemo-globin in urine	Phenyl-hydra-zine
	grams per 100 cc.	grams per 100 cc.	millions per cmm.	per cent	thous-ands per cmm.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		grams
1	22.7		10.2	0.8	19.3					
2	22.3		9.4	1.1	16.9					
3	22.3		9.7	0.6	16.5					
4	22.3		8.2	0.7	19.3					
5	24.0		9.2	0.6	14.3					
6	22.3	21.4	7.4	0.7	19.0					
7	22.7		7.5	0.7	18.2					0.2
8	22.3	20.3	7.9	2.6	20.5					0.3
9	22.3	20.7	10.0	10.5	20.6	14	1.3	2		0.3
10	22.3	20.1	9.2	9.9	17.7					0.3
11	22.3		10.0	10.5	22.2					0.3
12	21.0		7.7	5.8	23.1					0.3
13	21.5		9.8	4.2	25.3					0.3
14	21.0		6.5	3.5	18.9					
15	21.5		8.6	3.8	21.2					
16	20.4	20.8	8.4	5.2	19.9					
17	20.1		8.6	3.1	20.5					0.6
18	20.7	19.3	8.0	8.8	25.5					0.6
19	18.0	19.0	8.0	4.3	20.2			4		0.4
20	18.0		7.1	10.3	22.2					0.4
21	17.8	18.5	7.6	3.0	22.7				positive	0.4
22	17.4		7.1	3.5	38.5				positive	0.4
23	17.1		6.9	5.7	45.9			12	positive	0.4
24	15.8	15.1	6.3	4.4	40.0				positive	0.3
25	12.3		6.4	2.0	69.7	31	1.2	9	positive	
26	11.5	12.2	6.0	3.5	83.0				positive	
27	10.4		4.6	5.0	83.0			13	positive	
28	10.1	10.1	4.2	2.1	73.0				positive	

TABLE I (*continued*)

Day	Hemo-globin, New-comer method	Hemo-globin, iron method	Erythro-cytes	Reticu-locytes	Leuko-cytes	Blood urea nitro-gen	Blood uric acid nitro-gen	Blood bili-rubin	Hemo-globin in urine	Phenyl-hydra-zine
	grams per 100 cc.	grams per 100 cc.	millions per cmm.	per cent	thous-ands per cmm.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.		grams
29	9.6		4.1	4.6	94.1	64	1.7	14	positive	
30	9.6	9.4	3.9	1.1	71.1					
31	7.6		3.4	2.8	60.5			12		
32	7.5	7.2	3.8	8.2	53.0					
33	7.4	7.0	3.2	5.6	47.9					
34	7.6		2.6	4.6	41.2	51	2.1	12		
35	7.9		2.9	4.2	33.9					
36	8.4		3.0	4.1	42.0					
37	7.9	6.8	3.1	3.9	28.5			6		
38	7.6		3.1	6.7	33.8					
39	7.5	6.2		5.1	27.7					
40	7.6		3.1	3.9	20.6					
41	7.8		3.3	6.1	18.3					
42	7.9		3.0	5.6	19.1					
43	8.4	8.3	3.6	5.5	13.6	28	1.9			
44	8.5	8.7	3.5	3.8				3		
45	9.9		3.2	3.5	16.4					
46	9.7	10.1	3.7	4.1	19.3					
47	9.9		3.3	5.9	15.5					
48	10.3	11.3	3.5	3.7	8.6	21	1.9			
49										
50	10.1			3.6						
51			4.4		20.4					
52										
53	10.4	13.5	4.2	1.8	28.0					
54										
55										
56		13.8								
57										
58	13.2		4.8	3.1	16.9					

showed inverted T waves in all leads. There was neither cardiac pain nor congestive heart failure during the period of observation. Erythrocyte counts as high as 10,000,000 per cu. mm. had been obtained before the patient's admission. The patient cooperated in every respect and no medication was given except acetyl phenylhydrazine.

DISCUSSION

Observation of the charts will show that in both cases there was a very definite increase in oxygen consumption coincident with the negative nitrogen balance which followed the administration of phenylhydrazine. Both blood destruction and increase in oxygen consumption made their appearance several days after phenylhydrazine was first administered. Under

these circumstances it seems unlikely that the change in basal metabolic rate was due to a direct stimulating action of the drug upon the oxidative processes of the body cells. The data in these cases of polycythemia show a relationship between oxygen consumption and nitrogen metabolism which is similar to that previously described in pernicious anemia and in leukemia. In all these diseases the oxygen consumption is increased when there is an increase in endogenous protein catabolism.

The respiratory quotient did not change significantly during the periods of blood destruction. The body weight in Case 1 remained constant while that of Case 2 increased slightly more than one kilogram during the period of metabolic study. The mouth temperature in Case 2 remained constant throughout the period of study. There was a slight elevation of the tem-
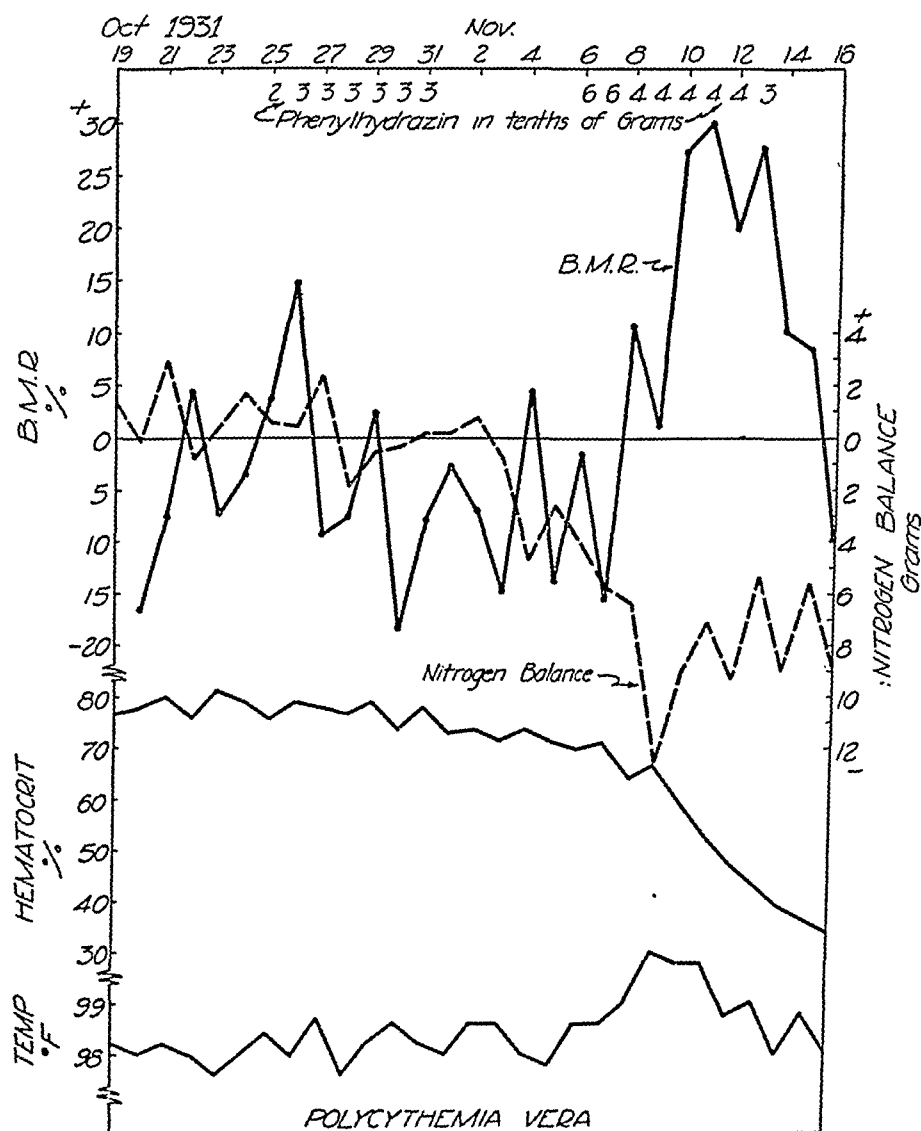
FIGURE 1

perature in Case 1 during the period of greatest nitrogen loss. This period also corresponded with the greatest increase in oxygen consumption, but the fever was insufficient to account for all of the increase in heat production. The respiratory rate varied between 12 and 20 per minute in Case 1 and between 12 and 16 per minute in Case 2. The pulse rate reached 112 per minute once in Case 1 and on four occasions it was found to be 100 per
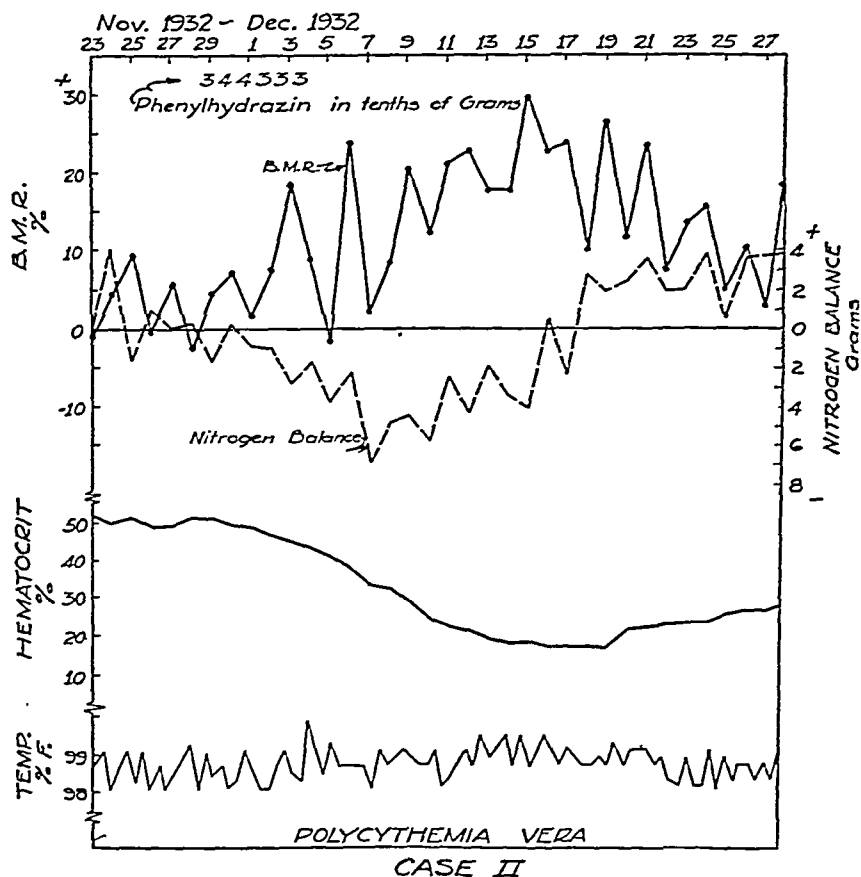
FIGURE 2

minute. In Case 2 the pulse rate did not exceed 84 per minute. The pulse and respiratory rates were determined just before and during the determination of basal metabolic rate. For a discussion of the effect of changes in the pulse and respiratory rates the reader is referred to a previous paper on leukemia (2).

The hemoglobin as determined by the Newcomer method corresponded quite well with the amount of hemoglobin as calculated from the iron content of the blood. This was true even when blood destruction was most

marked and the blood bilirubin values were quite high. Plasma iron determinations were not made. The fact that iron was not excreted in large amounts during the period of blood destruction might indicate that the iron derived from blood destruction either did not enter the circulation or was quickly removed. During the control period in Case 1 the hemoglobin averaged 22.6 grams per 100 cc., the erythrocyte count 9.0 million per cu. mm. and the corpuscle volume 78.5 per cent. After phenylhydrazine the hemoglobin decreased to 7.4 grams per 100 cc. and then increased to 13.2 grams at the end of the period of study. The corpuscle volume decreased to 28 per cent but was 45 per cent at the end of the period and the erythrocyte count decreased to 2.6 million per cu. mm. but reached 4.8 million per cu. mm. by the end of the period of observation.

In Case 2 the hemoglobin averaged 19.7 grams per 100 cc. during the control period; the corpuscle volume averaged 50.3 per cent and the erythrocyte count 6.5 million per cu. mm. During phenylhydrazine intoxication these values were reduced to 5.2 grams per 100 cc., 16.5 per cent and 1.6 million per cu. mm. respectively.

TABLE II

Data in Case 1

Period	Length of period	Average daily caloric intake	Average daily urea	Average daily uric acid	Average daily protein in urine	Average daily urinary nitrogen	Average daily fecal nitrogen	Average daily phosphorus balance*	Average daily urinary iron	Average daily fecal iron	Average daily iron balance*
	days	*calories*	*grams of nitrogen*	*grams of nitrogen*	*grams of nitrogen*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*
1	6	2120	8.12	0.081	0.07	10.20	0.85	+0.08	1.6	11.0	−4.3
2	7	1970	8.00	0.079	0.07	10.08	0.93	+0.07	1.3	13.5	−7.4
3	3	1924	8.11	0.075	0.06	9.98	0.39	+0.43	0.8	12.1	−6.2
4	6	1708	10.68	0.117	0.25	13.16	1.32	+0.05	2.1	14.1	−10.3
5	6	1585	11.13	0.116	0.49	13.41	0.37	+0.50	7.8	7.0	−10.1
6	9	1691	9.10	0.111	0.11	11.32	0.94	−0.06	1.7	15.9	−12.3
7	7	1545	3.77	0.064	0.07	4.83	0.61	+0.03	0.8	8.1	−4.1
8	7	1764	3.38	0.097	0.05	4.64	1.02	+0.13	0.6	17.1	−12.0
9	7	2134	4.01	0.080	0.05	5.38	0.54	+0.39	0.9	7.7	−2.1

* The amount of phosphorus and iron ingested was calculated from Rose's tables (26).

In both cases the reticulocytes increased during the period of blood destruction, in Case 1 to 10.5 per cent and in Case 2 to 22.6 per cent. The reticulocyte increase varied but persisted for many days. This observation is hard to reconcile with the impressions gained from extensive clinical use of the drug by Giffin and Allen (22), in that these authors thought that phenylhydrazine retarded blood formation. If Jackson's (23) observations that the products of leukocytic destruction stimulate leukopoiesis and if the clinical impressions that the injection of hemoglobin or whole blood

is followed by erythropoiesis are correct, then one might have anticipated an increased rate of erythropoiesis under the conditions of these experiments.

The circulating leukocytes increased to 94,100 per cu. mm. in Case 1 and to 20,900 per cu. mm. in Case 2. The increase was principally in polymorphonuclear neutrophiles and on one occasion 2 per cent of myelocytes were found in the blood of Case 1. We found no evidence which could permit us either to affirm or to deny the supposition that the leukocytosis was a response to primary destruction of leukocytes. The leukocytosis which occurred in these cases might suggest the use of phenylhydrazine in neutropenic states but its effect on the erythrocytes is so great that such a procedure would seem impractical.

The urea nitrogen of the blood increased to 64.4 mgm. per 100 cc. in Case 1 and to 17.5 mgm. per 100 cc. in Case 2 during the period of blood destruction. A slight increase was found in the uric acid nitrogen of the blood in Case 1 but the blood creatinine values were practically unchanged in both. These findings would indicate that under the conditions of this experiment urea can be formed in the liver more rapidly than it is excreted. The difference in the two patients may be one of renal efficiency. Also one might conclude that the urea forming function of the liver is not greatly damaged by phenylhydrazine, even in the excessively large doses which we used.

Blood bilirubin was much increased during blood destruction and the pigment gave a biphasic van den Bergh reaction in Case 1. The highest values obtained were 14.0 and 5.3 mgm. of bilirubin per 100 cc. of plasma

TABLE III

*The amount of urinary iron contained in precipitable protein in the presence of hemoglobinuria * (Case 1)*

Day	Urinary iron	Iron in filtrate	Iron in precipitate
	mgm.	*mgm.*	*mgm.*
21	2.10	1.46	0.50
22	6.67	3.17	3.47
23	10.17	7.17	2.59
24	7.29	5.80	1.89
25	10.09	4.63	5.81
26	9.24	4.34	4.90
27	6.38	4.93	1.19
28	3.71	3.12	0.55

* On the days in which there was hemoglobinuria, as shown by a chemical test (20) for blood, the urine was treated with trichloracetic acid, and iron determinations were made separately on the filtrate and on the precipitate. On the twenty-ninth day the amount of hemoglobin was very small and was not measured.

in Cases 1 and 2 respectively.   Here again we must assume that bilirubin can be formed more rapidly than it is excreted by the liver.

In one patient (Case 1) hemoglobin appeared in the urine and in both cases the urine was highly pigmented during the period of greatest blood destruction.   A positive Schlesinger's test for urobilin in the urine was obtained on 21 of the 58 days of observation in Case 1 and in all except 2 of the 36 days in Case 2.   Certainly there was no definite correlation between the amount of urobilin in the urine and the rapidity of the blood destruction.   In both cases positive tests for urobilin in the urine were obtained during the control period.   The total amount of pigment in the urine seemed to parallel the destruction of blood.

The excretion of phosphorus after phenylhydrazine did not correspond closely to the nitrogen excretion as was the case in our patients with leukemia who were treated with roentgen ray (2).   Both patients remained approximately in phosphorus equilibrium.   Similar findings have been recorded by Bassett, Killip and McCann (24) who have discussed a possible explanation for the failure of these patients to lose phosphorus during phenylhydrazine intoxication.

Bassett, Killip and McCann as well as Reznikoff (25) have demonstrated that very little if any of the hemoglobin iron liberated by phenylhydrazine intoxication is lost from the body.   In this regard our findings are in accord with those of the investigators previously mentioned. Whereas Bassett and his co-workers found a slight retention of iron during the period of greatest blood destruction, the patient reported by Reznikoff as well as both of ours suffered slight losses of iron.   Although the amount of iron in the feces of our Case 1 varied from one period to another, we do not feel that our findings lend support to the supposition of Bassett and his co-workers that food iron is better absorbed during rapid blood destruction than at other times.   In fact, the small amount of iron which our Case 2 lost during blood destruction was accounted for principally by an increase of iron in the feces.   The urinary iron was increased in Case 1 during the period of greatest blood destruction (Table II).   During control periods the average excretions of iron in the urine were 1.6 and 1.3 mgm. daily, while the urinary iron values exclusive of hemoglobin iron averaged 5.1 mgm. daily during the period of greatest blood destruction. During the immediately subsequent periods the urinary iron diminished as is shown by average daily excretions of 1.7, 0.8, 0.6, and 0.9 mgm., respectively, for four periods of about 7 days each.   In Case 2 the average amount of urinary iron was very close to 1.0 mgm. daily.   No significant variation in this amount occurred during the periods of blood destruction and subsequent erythropoiesis.

The excretion of creatinine in the urine was not materially changed during the period of blood destruction in either patient.   The same was true of the excretion of uric acid and ammonia.   There was a slight in-

TABLE IV

Data in Case 2

Hemo-globin, Newcomer method	Hemo-globin, iron method	Erythro-cytes	Reticu-locytes	Leuko-cytes	Blood urea nitro-gen	Blood uric acid nitrogen	Blood bilirubin	Phenyl-hydrazine
grams per 100 cc.	*grams per 100 cc.*	*millions per cmm.*	*per cent*	*thousands per cmm.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*grams*
18.9	19.6		2.5		14	1.7	0.2	
19.2		6.7	2.3	10.7				
19.5			2.0		14	1.8	0.3	
20.7		6.1	3.0	10.8				
19.8	19.8		2.6					
20.1		6.8	2.8	9.4				
18.9	18.9		2.5					0.3
18.0		6.1	3.2	10.2	14	1.7	0.1	0.4
17.4	17.2		1.3		11	1.4	0.4	0.4
16.3		5.4	1.8	11.5				0.3
15.7	15.0	5.1	2.0	8.6	11	1.4	1.3	0.3
14.9		5.3	2.5	10.6				0.3
14.2	14.1		1.7					
13.9		4.2	2.4	9.7				
13.1	12.7		3.6		15	1.1	3.3	
12.4		3.6	2.9	15.2				
10.8	10.1		2.8					
9.9		3.2	2.5	11.7				
8.7	8.8		2.6					
7.2		2.5	3.0	15.4				
7.3	6.8		3.9					
6.3		2.3	6.5	19.4	18	1.3	5.3	
5.7	5.8		22.6					
5.8		2.1	15.3	14.6				
5.5	5.4		13.7					
5.4		1.7	9.6	20.9				
5.3	5.6	1.6	15.2	9.4				
6.4		2.3	19.9	11.0				
6.8	5.8	2.2	18.2	9.5				
7.2		2.2	17.8	7.7				
7.2	7.7		11.2					
7.5		2.2	8.2	5.9				
7.7	8.3		7.9					
7.8		3.0	6.4	9.8				
7.9	8.4		5.1					
8.3		3.0	8.2	6.9	11	1.4	0.2	

in proteinuria in both patients during the period of blood destruc-
Most of the increase in nitrogen excretion was accounted for by a
efinite increase in urea in the urine. This observation was also made
ffman (10). The marked increase in urea excretion without a cor-
ding increase in the excretion of uric acid indicates that in these

experiments it was not catabolism of nucleoprotein which caused the increase in oxygen consumption. In leukemia (2) an increase in endogenous nitrogen catabolism was induced by irradiation of the splenic area. This procedure caused a moderate increase in uric acid excretion along with a considerable excess excretion of urea, and was accompanied by an increase in oxygen consumption. However, these observations do not prove that the catabolism of nucleoprotein is unimportant in causing an increase of basal metabolism such as that which is frequently observed in untreated cases of polycythemia vera.

TABLE V

Data in Case 2

Period	Length of period	Average daily caloric intake	Average daily urea	Average daily uric acid	Average daily protein in urine	Average daily urinary nitrogen	Average daily fecal nitrogen	Average daily phosphorus balance	Average daily urinary iron	Average daily fecal iron	Average daily iron balance
	days	*calories*	*grams of nitrogen*	*grams of nitrogen*	*grams of nitrogen*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*
1	6	2916	10.16	0.145	0.12	12.24	1.09	+0.27	0.90	7.67	+4.1
2	6	2765	11.44	0.156	0.12	13.56	1.16	+0.20	1.06	10.77	−0.4
3	6	2822	14.52	0.142	0.14	17.16	1.25	+0.61	0.96	19.67	−8.9
4	6	2702	12.33	0.158	0.14	14.44	1.30	+0.77	0.85	16.00	−5.6
5	6	2726	7.97	0.174	0.15	10.32	1.12	+0.35	1.00	15.67	−6.3
6	6	2686	6.96	0.212	0.18	9.30	1.00	+0.24	1.03	11.67	−2.4

There was a very profound decrease in the relative viscosity of the blood of both patients. In Case 1 the readings were 15.3 and 2.2, before and after phenylhydrazine, and in Case 2, 10.7 and 3.1 respectively. Readings of about 4.5 were obtained when normal blood was subjected to the same technique. Such a decrease in viscosity must result in a diminution of the cardiac work of such patients. Judging by our calculations in a previous paper (2) we would not expect this change in cardiac work to cause a significant change in the total metabolism of the patient.

By measurement of the diameter of the erythrocytes and by calculation of the volume indices, it appears that slightly larger erythrocytes are found following the hemolysis caused by phenylhydrazine. In neither case did the blood smear resemble that of pernicious anemia and the color index did not show a definite increase.

In Case 2 the blood calcium remained within normal limits as did the inorganic phosphorus. The total phosphorus of whole blood diminished as the erythrocyte count decreased but the lipid phosphorus fraction remained nearly constant. The serum albumin decreased from 4.9 per cent in the control period to 3.2 per cent after phenylhydrazine. The serum globulin increased from 2.1 per cent during the control period to 3.6 per cent after phenylhydrazine.

SUMMARY

Phenylhydrazine administered to two patients with polycythemia vera effected a number of metabolic changes.

1. A temporary negative nitrogen balance developed which was referable to a marked increase in excretion of urea and a slight increase in proteinuria together with a decrease in the nitrogen intake.

2. The variations of total oxygen consumption observed were parallel to the changes occurring in the endogenous nitrogen catabolism.

3. The relatively huge quantity of iron liberated by destruction of erythrocytes was nearly all retained in the body.

4. The rapid destruction of blood by phenylhydrazine was attended by an increase in circulating reticulocytes.

5. Both urea and bilirubin were formed more rapidly than they were excreted.

6. The patients remained approximately in phosphorus balance.

7. Case 2 showed a moderate leukocytosis and Case 1 a very marked leukocytic response to phenylhydrazine.

8. Urobilin appeared in the urine of both patients at frequent intervals. This pigment did not appear to be much more concentrated in the urine during periods of blood destruction than at other times. Hemoglobin appeared in the urine of Case 1. Most of the urinary pigment during blood destruction was unidentified.

9. The blood viscosity decreased greatly during the period of blood destruction.

10. In Case 2 the albumin-globulin ratio dropped from 2.3 to 0.9 during the period of blood destruction.

11. The average size of the erythrocytes increased but slightly during the period when hemoglobin ingredients were probably most plentiful.

12. The doses of phenylhydrazine which were employed are too large for routine clinical use.

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TABLE V

Data in Case 2

Period	Length of period	Average daily caloric intake	Average daily urea	Average daily uric acid	Average daily protein in urine	Average daily urinary nitrogen	Average daily fecal nitrogen	Average daily phosphorus balance	Average daily urinary iron	Average daily fecal iron	Average daily iron balance
	days	*calories*	*grams of nitrogen*	*grams of nitrogen*	*grams of nitrogen*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*
1	6	2916	10.16	0.145	0.12	12.24	1.09	+0.27	0.90	7.67	+4.1
2	6	2765	11.44	0.156	0.12	13.56	1.16	+0.20	1.06	10.77	−0.4
3	6	2822	14.52	0.142	0.14	17.16	1.25	+0.61	0.96	19.67	−8.9
4	6	2702	12.33	0.158	0.14	14.44	1.30	+0.77	0.85	16.00	−5.6
5	6	2726	7.97	0.174	0.15	10.32	1.12	+0.35	1.00	15.67	−6.3
6	6	2686	6.96	0.212	0.18	9.30	1.00	+0.24	1.03	11.67	−2.4

There was a very profound decrease in the relative viscosity of the blood of both patients. In Case 1 the readings were 15.3 and 2.2, before and after phenylhydrazine, and in Case 2, 10.7 and 3.1 respectively. Readings of about 4.5 were obtained when normal blood was subjected to the same technique. Such a decrease in viscosity must result in a diminution of the cardiac work of such patients. Judging by our calculations in a previous paper (2) we would not expect this change in cardiac work to cause a significant change in the total metabolism of the patient.

By measurement of the diameter of the erythrocytes and by calculation of the volume indices, it appears that slightly larger erythrocytes are found following the hemolysis caused by phenylhydrazine. In neither case did the blood smear resemble that of pernicious anemia and the color index did not show a definite increase.

In Case 2 the blood calcium remained within normal limits as did the inorganic phosphorus. The total phosphorus of whole blood diminished as the erythrocyte count decreased but the lipid phosphorus fraction remained nearly constant. The serum albumin decreased from 4.9 per cent in the control period to 3.2 per cent after phenylhydrazine. The serum globulin increased from 2.1 per cent during the control period to 3.6 per cent after phenylhydrazine.

SUMMARY

Phenylhydrazine administered to two patients with polycythemia vera effected a number of metabolic changes.

1. A temporary negative nitrogen balance developed which was referable to a marked increase in excretion of urea and a slight increase in proteinuria together with a decrease in the nitrogen intake.

2. The variations of total oxygen consumption observed were parallel to the changes occurring in the endogenous nitrogen catabolism.

3. The relatively huge quantity of iron liberated by destruction of erythrocytes was nearly all retained in the body.

4. The rapid destruction of blood by phenylhydrazine was attended by an increase in circulating reticulocytes.

5. Both urea and bilirubin were formed more rapidly than they were excreted.

6. The patients remained approximately in phosphorus balance.

7. Case 2 showed a moderate leukocytosis and Case 1 a very marked leukocytic response to phenylhydrazine.

8. Urobilin appeared in the urine of both patients at frequent intervals. This pigment did not appear to be much more concentrated in the urine during periods of blood destruction than at other times. Hemoglobin appeared in the urine of Case 1. Most of the urinary pigment during blood destruction was unidentified.

9. The blood viscosity decreased greatly during the period of blood destruction.

10. In Case 2 the albumin-globulin ratio dropped from 2.3 to 0.9 during the period of blood destruction.

11. The average size of the erythrocytes increased but slightly during the period when hemoglobin ingredients were probably most plentiful.

12. The doses of phenylhydrazine which were employed are too large for routine clinical use.

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THE EFFECT OF DIIODOTYROSINE ON THE BASAL METABOLISM IN MYXEDEMA [1]

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Much speculation has developed concerning the rôle of diiodotyrosine in the thyroid, particularly since the report of its isolation from the gland by Harington and Randall (1) in 1929. According to these authors, the iodine of the thyroid is about equally divided between diiodotyrosine and thyroxine and diiodotyrosine is to be regarded as the precursor of thyroxine. Observations on the biological effects of diiodotyrosine had been started some years before their report. Strouse and Voegtlin (2) in 1909 did not notice any clinical improvement following the administration of 0.1 gram three times a day for ten days and two weeks to a patient with myxedema and to a cretin respectively. Knipping and Wheeler-Hill (3) gave 100 mgm. of diiodotyrosine daily by mouth for several weeks to men and observed no effect on the basal metabolism, pulse, weight, or vegetative nervous system. Hoffmann (4) gave 3, 5, diiodo-1-tyrosine subcutaneously to normal men in total doses varying from 100 mgm. in one day to 8.4 grams in fourteen days and he likewise observed no effect on the basal metabolism. Beumer and Kornhuber (5) gave 19.5 grams of diiodotyrosine over an eight-day period to a four year old child and noted no influence on diuresis or weight. In a two year old cretin, who had improved greatly on thyroid, a relapse occurred when they gave from 0.5 to 1.0 gram of diiodotyrosine three times daily for eight weeks. The only contradictory observation appears to be that of Abderhalden (6), who reported beneficial results from feeding 3, 5, diiodo-1-tyrosine to three children (siblings) who all showed well marked symptoms of myxedema.

As in the case of man, the data reported on lower animals do not furnish satisfactory evidence of a thyroid-like action of diiodotyrosine. Strouse and Voegtlin (2) observed that it was without effect on the nitrogen metabolism and blood pressure in dogs. Abelin (7) and Gaddum (8) reported that it did not increase the metabolism of normal rats. Abderhalden and Wertheimer (9) showed that while thyroxine reduced the weight rapidly in rabbits and guinea pigs, diiodotyrosine had only a

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and minus 26 per cent at the end of the period of injection. However, if only the figures at the beginning and end of the injections of diiodotyrosine are considered, then the metabolism did increase from minus 34 per cent to minus 26 per cent and the patient lost about 1.5 kgm. in weight during this period. These changes may be of no significance, they may
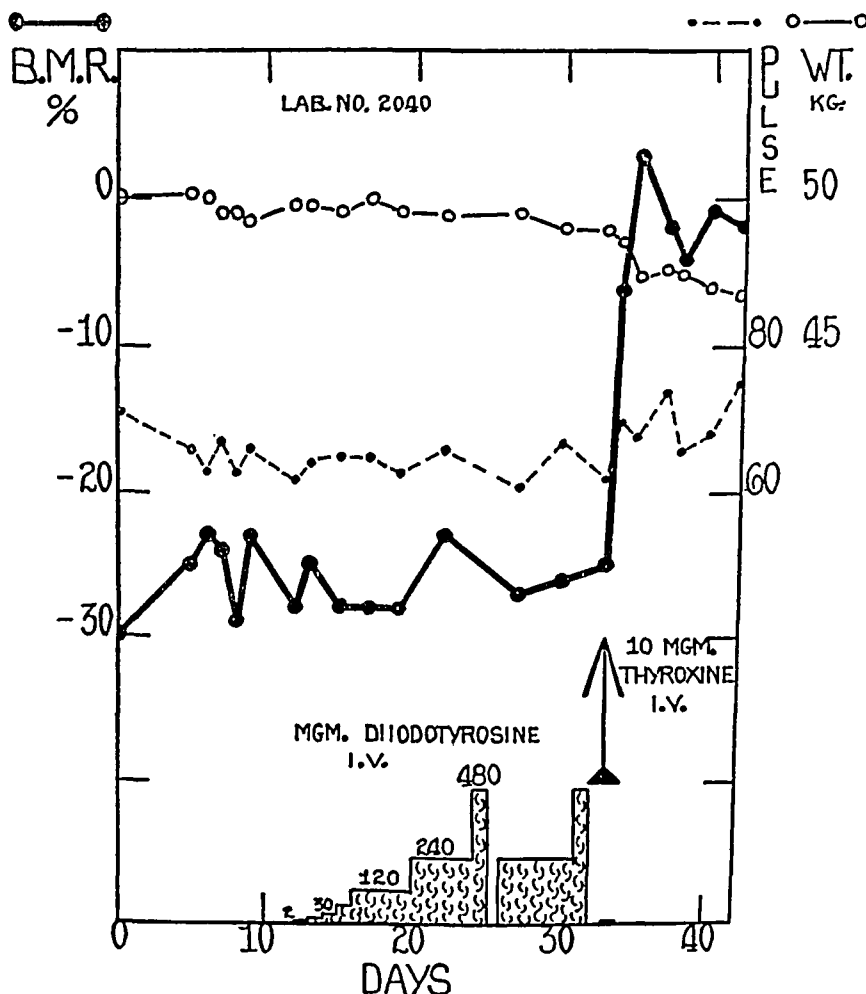
CHART 1. MRS. M. K. LABORATORY NUMBER 2040. HEIGHT 152 CM. AGE 36

Showing no change in basal metabolism during the intravenous administration (i.v.) of large doses of diiodotyrosine to a patient with spontaneous myxedema and the prompt increase following the intravenous administration of a small dose of thyroxine (arrow).

have been caused by the omission of compound solution of iodine, or they may represent a slight effect from diiodotyrosine. In contrast with the lack of a definite effect from diiodotyrosine is the prompt rise in metabolism following the injection of a relatively minute amount of thyroxine. Thus, in the first patient, 10 mgm. of synthetic thyroxine (Hoffmann-La Roche) injected intravenously caused an increase in the metabolism from

minus 26 per cent to minus 2 per cent and in the second patient, the intra-
venous injection of 7.5 mgm. of thyroxine caused an increase in basal
metabolism from minus 26 per cent to minus 7 per cent.   The iodine in
these doses of thyroxine was only one three-hundred-and-thirty-sixth and
one six-hundred-and-eleventh respectively of that in the doses of diiodo-
tyrosine.
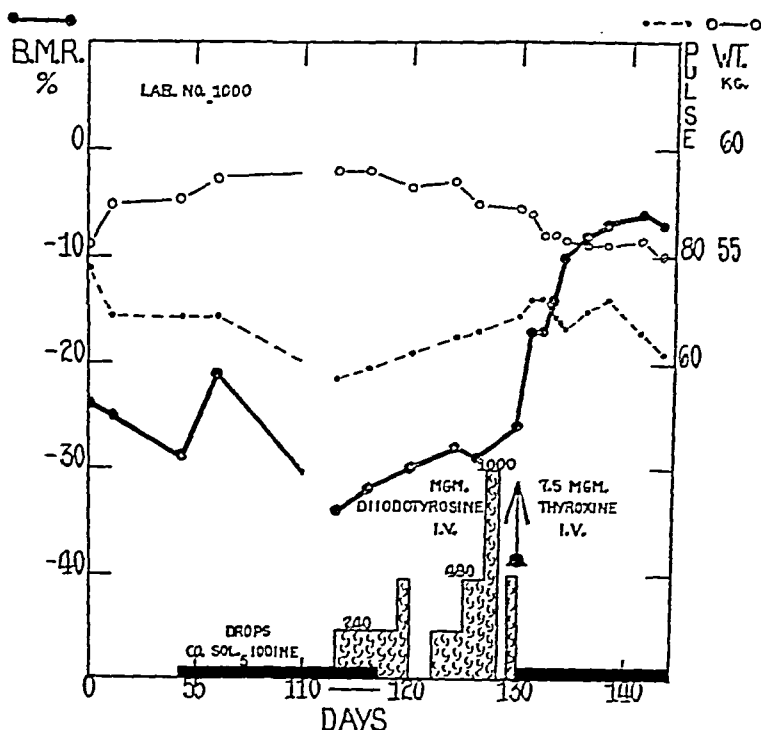
CHART 2.   MRS. A. R.   LABORATORY NUMBER 1000.   HEIGHT 160 CM.   AGE 33
    Showing no definite change in basal metabolism during the intravenous ad-
ministration of diiodotyrosine to a patient who developed myxedema following
a thyroidectomy and the prompt increase following the intravenous administra-
tion of a small dose of thyroxine (arrow).

<div align="center">COMMENT</div>

    Since the basal metabolism in the most marked cases of myxedema is
from 40 to 45 per cent below the normal and since it was depressed only
26 per cent and 28 per cent below normal in the subjects of this study, it
is not improbable that they both had a small amount of functioning thyroid
tissue, although none could be felt in either.   It is probable that this tissue
could affect our results in only two ways: (1) By storing some diiodo-
tyrosine.   (2) By using it to form more thyroxine.   Even if the normal
amount of thyroid tissue were present, the maximum storage capacity for

Details of administration of diiodotyrosine to two patients with myxedema

Case 1		Case 2	
Date	Amount of diiodotyrosine injected intravenously	Date	Amount of diiodotyrosine injected intravenously*
1932	*mgm.*	*1932*	*mgm.*
July 18	2.1	July 30	240.0
19	10.8	August 1	240.1
20	30.2	2	240.4
21	58.9	3	240.3
22	134.6	4	240.2
23	120.0	5	240.5
24	121.6	6	480.5
25	121.1	8	240.2
26	241.1	9	240.5
27	240.3	10	240.0
28	240.8	11	481.0
29	240.7	12	480.1
30	480.3	13	1000.6
August 1	240.0	15	500.1
2	241.1		
3	240.6		
4	240.1		
5	240.5		
6	480.3		
Total	3725.		5105.

* Compound solution of iodine (5 drops daily), was administered from May 26, 1932 to August 2, 1932. Administration of this compound was started again August 16, 1932.

iodine would have been 25 mgm. or about 43 mgm. of diiodotyrosine. Since the metabolism remained almost stationary, it appears that the output of thyroxine was neither increased nor decreased by the enormous excess of diiodotyrosine administered. In other words, the limiting factor in the output of thyroxine was not the supply of diiodotyrosine; and, as the data on the second patient show, apparently not the supply of iodine. From the observations of Knipping and Wheeler-Hill (3), it appears that a similar conclusion applies to the normal thyroid gland.

If it be true, as Harington and Randall (1) claim, that diiodotyrosine is the precursor of thyroxine, then two conclusions may be drawn from our data: (1) Diiodotyrosine probably cannot affect the metabolism without being synthesized into thyroxine. (2) This synthesis does not appear to take place in the tissues of the body outside of the thyroid gland. Our data, together with those of Gaddum (8) reported above, serve to emphasize the importance of preserving the integrity of the thyroxine molecule if its characteristic results are to be produced. Even the work on tadpoles

would appear to support this conclusion, because of the enormous quantity of other substances required to cause metamorphosis in contrast with the relatively minute amounts of thyroxine. The reason for the apparent difference in the effect of very large doses of diiodotyrosine in tadpoles and man is not clear. Means, Lerman and Salter (27) have recently advanced the hypothesis that diiodotyrosine assumes calorigenic properties when linked with amino-acids in iodothyreoglobulin.

SUMMARY

In one patient with myxedema the administration of 3.7 grams of diiodotyrosine in nineteen intravenous injections over a period of 21 days was without effect on the basal metabolism; and in another patient with myxedema (who was also receiving compound solution of iodine for the first four days) the administration of 5.1 grams in fourteen intravenous injections over a period of 15 days was without a definite effect on the basal metabolism.

In patients with a small amount of functioning thyroid tissue the limiting factor in the output of thyroxine does not appear to be the supply of diiodotyrosine.

It would appear that diiodotyrosine cannot be synthesized into thyroxine outside of the thyroid gland.

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its blood supply." They showed clearly that the pain is not due to arterial spasm. They demonstrated that lack of oxygen is not the direct cause of the pain but considered that it is quite probably an indirect cause. The pain is attributed to "a chemical or physico-chemical stimulus developed in the muscle mass during its exercise." This stimulus is termed a pain factor or factor P. They believe it is the accumulation of this P factor that causes the pain of intermittent claudication and of angina pectoris.

Reid (13) working in the laboratory of MacWilliam, made further observations on muscle pain. He states, "The abrupt disappearance of the pain (within a few seconds after readmission of blood) points to a chemical cause, and, more particularly, to the influence of unoxidized products of muscular activity on the sensory nerve-endings."

Keefer and Resnik (14) on theoretical grounds concluded that the anginal pain occurring in ischemia of the myocardium is to be attributed to the associated anoxemia. Recently, Rothschild and Kissin (15) and Dietrich and Schwiegk (16) were able to precipitate attacks of precordial pain by inducing a generalized anoxemia in patients presenting a history of attacks of angina pectoris.

The present investigation was designed to determine whether anoxemia without ischemia would lead to pain in healthy exercising skeletal muscles.

OBSERVATIONS

Eight young normal subjects having no evidence of peripheral vascular disease were studied. A progressive generalized anoxemia was induced by rebreathing from a 20 liter tank connected in series with an 8 liter spirometer; the accumulation of carbon dioxide was prevented by passing the expired air over soda-lime. A variable period (0 to 5 minutes) after starting rebreathing the subject began to squeeze an ergograph (Lewis (12)) at a definite rate set by a metronome (12 to 60 times per minute). The period of anoxemia was ended when the subject indicated any discomfort or when he became extremely cyanotic. The exercise, however, was continued at the set rate for a further period of several minutes. At the end of this experiment the subject described the sensations experienced. In six of the eight subjects psychic bias was precluded because the subjects were ignorant of the nature of the experiment. At the end of the anoxemic period a sample of expired air was drawn from a tap close to the subject, and the oxygen and carbon dioxide content determined by analysis with a Haldane apparatus.

As a control, each subject while breathing room air repeated his exercise at the same rate and as nearly as possible in the same position as in the anoxemia experiments. The sensations experienced by the subject during the control period were compared with the sensations during anoxemia.

The results are summarized in Table I. It will be noted that pain appeared in the exercising muscles in all eight subjects of the anoxemia ex-

TABLE I

Effect of generalized anoxemia on the production of pain in exercising muscle

Name	Oxygen level reached	Duration of rebreathing	Preliminary rebreathing period before start of exercise	Duration of exercise	Rate of exercise	Pain during exercise	Pain relieved by stopping rebreathing	Pain relieved by stopping exercise
	volumes per cent	*minutes*	*minutes*	*minutes*	*per minute*			
1. S. P.	6.5	14½	2	18	15	Yes	Yes	
	Room air			15½	30	No		
2. M. K.	7.6	14	5	9	12	No		
	6.2	13	0	14	30	Yes	Yes	
	Room air			23	30	No		
3. D. A.	10.0	11	4	9	30	Yes	Lessened	Yes
	Room air			15	30	No		
4. L. K.	10.0	10	0	11	30	Yes	Yes	
	Room air			15	30	No		
5. R. H.	11.4	10	2½	10	30	Yes	Yes	
	Room air			15	30	No		
6. K. J.	6.9	14	2	14	30	No		
	7.25	11	1	12	60	Yes	No	Yes
	Room air			15	60	No		
7. L. N. K.	7.1	10½	2½	10½	30	Yes	Yes	
	Room air			15	30	No		
8. L. R.	7.9	14	2	15	30	Yes	No	Yes
	Room air			15	30	Yes		Yes

periments. The pain disappeared with the termination of anoxemia in four subjects; in two the pain lessened in intensity, and in the other two it remained unabated. In these last four subjects termination of the exercise led to a disappearance of the pain. In three further experiments, the exercise was stopped after pain had developed for a short time. It was found that the pain diminished slightly and did not disappear until the subject again breathed room air.

The pain in all experiments was usually limited to the muscles of the forearm. Occasionally it was also felt in the flexor muscles of the hand. It began as a mild ache and became progressively more intense but never unbearable. The onset of the pain was gradual but with the termination of anoxemia its offset was sharp. The pain during anoxemia was not as severe as when the circulation was completely occluded in the four subjects in whom this comparison was made.

Seven of the eight subjects did not develop pain during exercise when breathing room air. This indicates that the generalized anoxemia was essential for the appearance of pain in the anoxemia experiments. The eighth subject developed pain during exercise in room air. In his case, some other factor than anoxemia must have been responsible for the appearance of pain. This other factor appears to be the rapidity at which the exercise is performed.

Other evidence that the rate of exercise is an important factor in the production of pain was observed. For example, K. J. (Number 6, Table I) experienced no pain during anoxemia when the exercise frequency was 30 times per minute, but developed pain when the exercise rate was doubled. Similarly, M. K. (Number 2, Table I) experienced no pain during anoxemia when exercising at the rate of 12 times per minute, but developed pain when the rate was increased to 30 times per minute.

In one subject breathing room air the effect of rate of exercise was studied in greater detail. The subject squeezed the ergograph at rates of 120, 80, 60, 30 and 15 per minute and the time of onset of mild, moderate and severe pain was noted. The results are recorded in Table II. It will

TABLE II

Time of onset of pain at various rates of exercise

Exercise rate	Mild pain	Moderate pain	Severe pain
per minute	*seconds*	*seconds*	*seconds*
120	60	70	85
80	95	115	180
60	185	225	none
30	none	none	none
15	none	none	none

be seen that the time at which pain developed was an inverse function of the rate of exercise. The more rapid the exercise rate, the sooner was the development of pain in the exercising muscles. The pain did not become severe even after five minutes when the rate of contraction was 60 times per minute and no pain appeared within fifteen minutes when the rate was 30 times per minute or slower. A similar result was obtained when the same subject breathed a mixture containing 11.4 volumes per cent of oxygen from a 70 liter Tissot spirometer. Exercise at the rate of 60 times per minute caused pain to develop after three minutes, but when the experiment was repeated with the rate of exercise reduced to 30 per minute no pain developed even after five minutes.

There appears to be an individual variation in the degree of anoxemia required to induce pain during exercise. The oxygen levels of the inspired air at the termination of the anoxemia experiments ranged from 6.2 to 11.4 volumes per cent (Table I).

One subject was studied in greater detail with regard to the effect of varying degrees of generalized anoxemia on the length of time required for the onset of pain. The subject breathed from a Tissot spirometer of 70 liters capacity filled with mixtures of nitrogen and oxygen in varying proportions. The gases were thoroughly mixed by connecting an anaesthesia bag to the outlet pipe of the spirometer and moving the bell up and down 20 times. In each experiment the oxygen content of the inspired air

was maintained at a fixed level. The subject was kept in ignorance of the proportion of gases in the mixture and the variations in the proportions in the several tests were made non-progressive to obviate psychic influences. Oxygen mixtures ranging from 20.6 to 8.4 volumes per cent were used and the exercise was also repeated with the patient breathing room air and with the arm rendered ischemic by a tourniquet. Exercise was begun one minute after the subject started to breathe from the spirometer. The rate of exercise was kept constant at 60 per minute in all experiments.

The results are shown graphically in Figure 1. It will be seen that

FIG. 1. TIME OF ONSET AND SEVERITY OF PAIN IN RELATION TO THE VOLUME PER CENT OF OXYGEN IN THE INSPIRED AIR
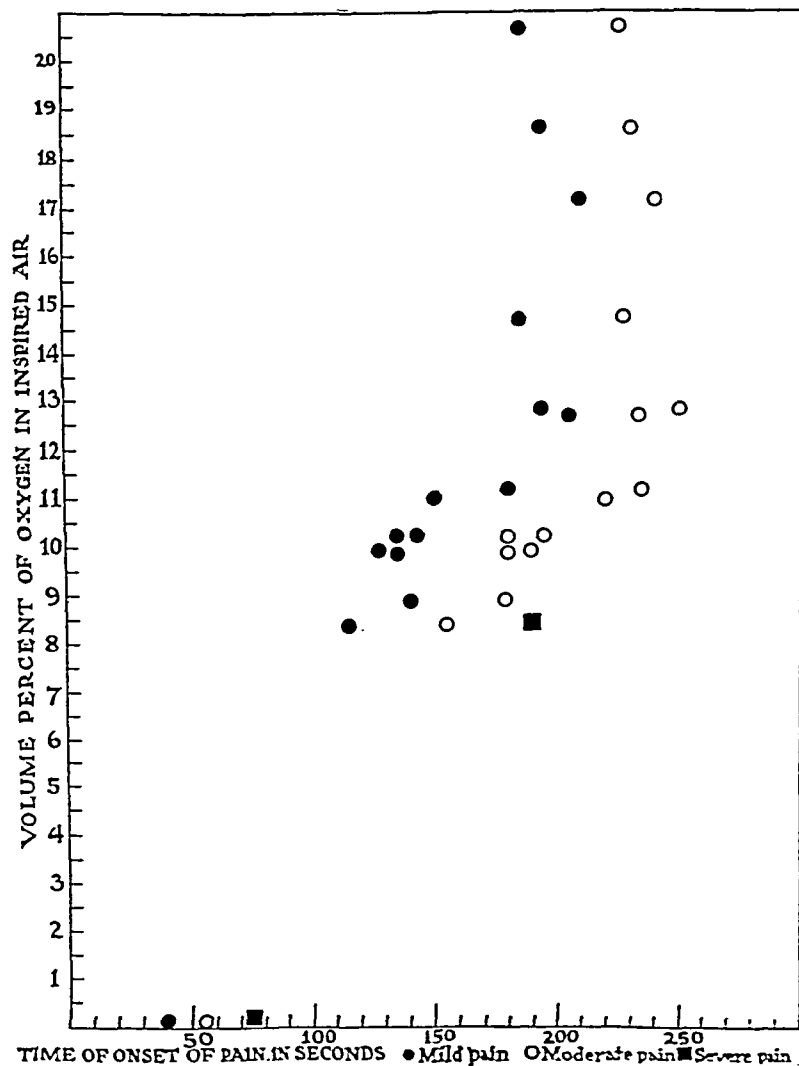
when the oxygen content of the inspired air was between 13 and 20.6 volumes per cent, the time of onset of pain was approximately constant. When the oxygen content was between 8.4 and 13 volumes per cent the time of onset varied, pain occurring earlier in the working muscles as the degree of anoxemia increased. Severe pain was absent until the oxygen level fell to 8.4 volumes per cent. Pain was most severe and came on most rapidly when the arm was rendered completely ischemic while the patient breathed room air.

Several observations on the effect of anoxemia upon the time of onset of pain were made at other exercise frequencies in the same subject. The results, summarized in Table III, agree in showing that pain developed sooner when the exercise was performed during general anoxemia.

TABLE III

Effect of anoxemia on time of onset of pain

Exercise rate	Oxygen content of inspired air	Time of onset of pain		
		Mild pain	Moderate pain	Severe pain
per minute	*volumes per cent*	*seconds*	*seconds*	*seconds*
80	12.1	90	115	130
80	Room air	95	115	180
120	11.9	35	40	55
120	Room air	60	70	85

DISCUSSION

The results of these experiments indicate that (*a*) anoxemia can lead to pain in an exercising skeletal muscle, (*b*) an individual variation exists in the degree of anoxemia required to produce pain, (*c*) in a single individual after a sufficiently low oxygen level is reached, the onset and severity of pain vary directly with the degree of anoxemia. The results show further that (*a*) the rate of exercise is also an important factor in the induction of pain, (*b*) an individual variation exists in the rate of exercise necessary to initiate pain, (*c*) in a single individual the onset and severity of pain vary with the rate of exercise.

Anoxemia and rate of exercise, the factors in the production of pain, can be correlated on the following basis: During exercise certain intermediary products of metabolism are formed which are further altered in the presence of a normal oxygen supply. The rate of their formation will be a direct function of the rate of exercise (providing the force of each contraction is kept constant). The rate of exercise may become so rapid that even in the presence of a normal supply of oxygen these metabolic products cannot be oxidized sufficiently rapidly to prevent their concentration in the muscle tissues. If the exercise is continued at this rapid rate

these metabolic products will reach a concentration above the pain threshold and so pain will be initiated. This condition can be called relative anoxemia.

On the other hand, if the exercise rate be kept constant and the oxygen supply to the muscle be cut down, a stage will be reached in which the oxidation of the metabolic products formed during exercise cannot keep pace with their production. These metabolic products will accumulate, and if the exercise be continued, the threshold for the production of pain will again be exceeded.

Even with a diminished oxygen supply the rate of exercise may be so slow that the oxidative alteration of these products of metabolism can keep pace with their production. No accumulation of these products will ensue and a " steady state " will develop without the appearance of pain.

This theory explaining the foregoing observations is compatible with our present ideas of muscular metabolism. The cause of the pain may be lactic acid which Fletcher and Hopkins (17), Hill (18), Meyerhof (19) and others have shown is formed during exercise; it may be a compound of phosphorus, of creatine, or ammonia, which Eggleton and Eggleton (20), Fiske and Subbarow (21), Embden et al. (22 and 23), Lundsgaard (24 and 25) and others have shown to be formed during exercise; or it may be another product as yet undetermined. In this regard it may be wise to follow the lead of Lewis (1 and 12) and call the pain stimulus the P factor.[3]

These results suggest that while stasis may be a factor in the production of pain in ischemia, anoxemia is essential. The anoxemia may be complete, partial, or relative. The absence of pain during ischemia of the resting extremity, found by Lewis et al. (12) does not necessarily indicate that the metabolic products formed in a resting muscle are qualitatively different from those formed in an exercising muscle. The absence of pain in the resting muscle can perhaps be explained by the fact that its metabolism is a small fraction of that in an exercising muscle (19). The time needed to accumulate sufficient P factor in the ischemic resting muscle to reach the pain threshold would be longer than the interval when pain sensibility is lost in the limb. Lewis, Pickering and Rothschild (26) have shown that the application of a restricting cuff about the arm results in loss of pain sensibility after 40 minutes.

In conclusion, this study suggests that anoxemia is an important factor in the mechanism producing pain in contracting skeletal muscle. Anoxemia may also be an important factor in producing the pain in intermittent claudication and angina pectoris.

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[3] The assumption that a chemical product is responsible for the pain may be wrong but the arguments with slight modification will still hold if the pain was due to a physicochemical change.

SUMMARY

1. Generalized anoxemia without ischemia can induce pain in an exercising skeletal muscle.

2. Within certain limits the severity and rapidity of onset of the pain varies with the degree of anoxemia and with the rate of exercise of the muscles involved.

3. The pain appears to be due to the accumulation of products of muscular metabolism that require oxygen for their disposal.

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SUMMARY

1. Generalized anoxemia without ischemia can induce pain in an exercising skeletal muscle.

2. Within certain limits the severity and rapidity of onset of the pain varies with the degree of anoxemia and with the rate of exercise of the muscles involved.

3. The pain appears to be due to the accumulation of products of muscular metabolism that require oxygen for their disposal.

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FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI. THE DEVELOPMENT OF RESISTANCE TO FIBRINOLYSIS FOLLOWING ACUTE HEMOLYTIC STREPTOCOCCUS INFECTIONS

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In a recently published report (1) results were presented which demonstrate that broth cultures of hemolytic streptococci are capable of rapidly liquefying the fibrin-clot of normal human plasma. A description was given of the presence of fibrinolytic substances in cultures, as well as in sterile filtrates, and of the conditions of fibrin coagulation which influence the occurrence of liquefaction. The fibrinolysin was found, by the experimental method described, in every strain of hemolytic streptococcus isolated from patients. On the contrary, cultures of other species of bacteria, also derived from human beings, were, in comparable tests, unable to cause fibrinolysis.

This special characteristic of human strains of hemolytic streptococci is being investigated both with reference to its biochemical properties and also for the purpose of determining whether or not the fibrinolytic activity of the living organisms might be a factor in hemolytic streptococcus infections. The results of the chemical investigations will be subsequently published.

The purpose of this article is to describe the resistance to streptococcal fibrinolysis which develops in the blood of patients following acute infections with hemolytic streptococci, and to contrast the insusceptibility exhibited by the blood of these patients with the results of similar tests on the plasma of patients suffering from other bacterial infections.

In addition to the observations which have been made with the blood from patients, tests have been performed with the fibrin-clot of plasma from healthy normal adults.

A fourth group of cases, whose plasma has been similarly tested, consists of patients in the dispensary or hospital who either presented no abnormal physical findings at the time blood was withdrawn, or in whom the low grade chronic ailment was deemed least likely to influence the fibrinolytic test with which this report is concerned. This group, although not normal, has been utilized in an attempt to obtain tests with blood from individuals in different age groups, normal healthy representatives of which

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were not available. For the purpose of this article they are tentatively considered as normal.

A test consists in mixing oxalated plasma with living, full grown, broth cultures of hemolytic streptococci, and then inducing clot formation by the addition of $CaCl_2$. With the fibrin-clot from the plasma of certain individuals, liquefaction is completed in a few minutes. On the other hand, when the plasma of other patients is coagulated in the presence of active cultures, the resultant coagulum resists liquefaction completely or delays the process. The interval of time, therefore, between clot formation and clot dissolution is used as an index of susceptibility or resistance to the fibrinolytic principle of active cultures.

No attempt has been made to establish arbitrarily a rate of dissolution to serve as a sharp dividing line between normal and abnormal. Minor variations in the quantity of fibrin in a given sample of clotted plasma or in the amount of fibrinolysin in the cultures may change the time required for dissolution by several minutes. However, by using constant amounts of plasma and culture in all the tests, the limits of experimental error due to the uncontrolled quantitative variation just referred to, have been minimized to such a degree that they do not cause a variation in dissolution time of more than 10 to 15 minutes. When, therefore, the difference in the time of liquefaction between the fibrin-clot of a patient's blood and that of a known susceptible is proved to be a matter of several hours, the delayed rate has been considered significant. As an aid in interpreting the importance of the time element in fibrinolytic tests, the following classification has seemed practicable.

> Dissolution in less than 30 minutes—Highly susceptible.
> Dissolution in 30 to 60 minutes—Susceptible.
> Dissolution in 1 to 8 hours—Definite resistance.
> Dissolution in 8 to 24 hours—Marked resistance.
> No dissolution in 24 hours—Maximum resistance.

All experiments were arbitrarily terminated at the end of 24 hours in water bath at 37.5° C.

MATERIALS AND METHODS

For a more complete description of all the details, the reader is referred to the previous article (1). As formerly stated, the experimental conditions which best promote the occurrence of rapid fibrinolysis consist in mixing cultures with oxalated plasma *before* inducing clot formation.

Cultures. A strain of hemolytic streptococcus, designated Co., and known to be highly active in the production of fibrinolytic substances, has been employed in most of the tests. Fresh 18 to 24 hour cultures were utilized. Plain meat infusion broth, containing 0.05 per cent dextrose, was the most satisfactory medium. Occasionally broth cultures, kept in the icebox, were used for 2 to 4 days. When, by testing the old cultures with susceptible fibrin-clot, the rate of dissolution was delayed, a fresh culture was employed.

In several instances, the hemolytic streptococci isolated from a patient, were used in tests with the patients' plasma. In such observations, however, no specificity was demonstrable, and no special advantage was obtained by employing strains and plasma from the same patient.

Although sterile filtrates have been found to contain the fibrinolytic principle in large quantities, whole broth cultures were employed in all the tests reported in this communication.   Since the tests were designed to determine resistance to clot liquefaction, the more potent cultures have been used instead of filtrate, in order to make the test more stringent and exacting.

Anticoagulant. Potassium oxalate has been regularly employed in amounts of 0.02 gram of oxalate to 10 cc. of blood. A 2 per cent solution is made in distilled water. One cubic centimeter of this solution is placed in small bottles which are heated in a dry air sterilizer until all water has evaporated. Ten cubic centimeters of blood, immediately after withdrawal, are mixed with the dried powder.

Coagulant. 0.25 cc. of a 0.25 per cent solution of $CaCl_2$ in 0.85 per cent salt solution consistently clotted 0.2 cc. of oxalated plasma in 8 to 20 minutes. The solution of calcium chloride was sterilized by immersion in boiling water for 30 minutes.

In the experiments reported in this article samples of plasma were always tested within 24 hours of the time blood was withdrawn. This fact is important since coagulation with $CaCl_2$ is delayed or inhibited in oxalated plasma which is several days old.

Description of test. The following quantities of the various ingredients, which have been uniformly employed, constitute a " standard " test: 0.2 cc. of oxalated plasma is diluted with 0.8 cc. of physiological salt solution. To this 1 to 5 dilution of plasma, 0.5 cc. of broth culture of test organisms is added and well mixed. 0.25 cc. of 0.25 per cent solution of $CaCl_2$ is then added and well mixed. The tubes are immediately placed in water bath at 37.5° C. Solid coagulation usually occurs in 6 to 20 minutes; the average time is about 10 minutes. Solid coagulation is considered to be effected when the tubes can be inverted without affecting the solid form of clot which adheres to the bottom and sides of the tube; usually no fluid, or only a small drop, escapes from the solid clot on inversion of the tube.

The tubes are allowed to remain in the water bath under continual observation. Dissolution of the clot is recorded as being complete when all evidence of solid fibrin has disappeared, and the contents of the tube are completely fluid.

By recapitulation, a test is as follows:

0.2 cc. oxalated plasma + 0.8 cc. physiological salt solution + 0.5 cc. culture + 0.25 cc. $CaCl_2$.

All tests in which the plasma-clot was resistant to dissolution were arbitrarily terminated after 24 hours incubation.

Before proceeding with a description of the results of fibrinolytic tests obtained with the blood of patients, some idea of the " normal " rate of reaction may be derived from the tests performed with the fibrin-clot from the plasma of healthy adults and of the specially selected group of dispensary and hospital cases previously mentioned.

Thirty healthy adults were chosen from students, technicians, and members of the hospital staff. In this group it was possible to select those who were healthy at the time the test was made, and who gave no history

of acute infections during the past winter. The results are recorded in Table I.

TABLE I

Fibrinolytic tests with plasma-clot from the blood of normal, healthy adults

Individual	Age dec- ade	Sex	Time required for complete dissolution of plasma-clot*		Individual	Age dec- ade	Sex	Time required for complete dissolution of plasma-clot*	
			hours	*minutes*				*hours*	*minutes*
1	3rd	M		15	16	3rd	F		10
2	3rd	M		8	17	3rd	M		16
3	3rd	M		12	18	3rd	M		10
4	3rd	M		15	19	3rd	M		20
5	3rd	M		25	20	3rd	M		5
6	3rd	F	1	5	21	3rd	M		45
7	3rd	F		5	22	3rd	M	1	
8	3rd	M		40	23	4th	M		15
9	3rd	F	1	35	24	4th	M		10
10	3rd	M		15	25	4th	M	2	20
11	3rd	M		10	26	4th	M		15
12	3rd	M	4		27	4th	F		25
13	3rd	M	3		28	4th	F		10
14	3rd	M	1	15	29	4th	M		25
15	3rd	M	3		30	4th	M		10

* Repeated tests have been made with many of the specimens of blood. The average rate is recorded in the table.

The normal controls listed above were all in the 3rd and 4th decades of life. There are 24 males and 6 females. In this small series the plasma-clot of 70 per cent was liquefied in less than one hour; only 2 of these required longer than 30 minutes. The time of clot dissolution in the remaining 30 per cent ranged from 1 hour and 5 minutes to 4 hours. Variation in the rate of fibrinolysis exhibited by the blood of different healthy individuals cannot, as yet, be interpreted. Consideration of this point will be given later in this article when the results of repeated tests are described.

Table II contains the results of tests performed with the blood of 30 patients who were selected with special reference to age. The nature of their chronic illnesses is recorded in the table, and, where possible, a history of recent acute infections is noted. Fourteen of the patients are under 13 years of age, two are in the 5th decade of life, and the ages of the remaining fourteen range from 50 to 73 years.

The children included in Table II came to the Dispensary of the Harriet Lane Home for Children. Eight of these were receiving routine antiluetic treatment for congenital syphilis. Plasma was obtained from the others at the time blood was taken for the Wassermann reaction. The plasma-clot from seven of the children liquefied in less than 1 hour, and the dissolution time in the remaining seven ranged from 2 hours and 15

TABLE II

Fibrinolytic tests with the plasma-clot from young and old patients with chronic disorders

Patient	Age	Disease	History of recent acute infection	Dissolution of plasma-clot*	
	years			hours	minutes
1	9	Congenital syphilis	None		14
2	10	Congenital syphilis	Acute tonsillitis, T. and A. 4 months ago		10
3	5	None	None		12
4	5	?	?		18
5	11	Congenital syphilis	None		23
6	8	Congenital syphilis	"Catches cold easily"		27
7	3	Under-nutrition	None		28
8	12	Congenital syphilis	Enlarged cervical glands 2 months ago		50
9	8	Congenital syphilis	Acute tonsillitis 2 months ago	2	15
10	13	"Worms"	?	3	10
11	7	?	?	3	
12	9	Congenital syphilis	None	4	
13	5	Under-nutrition	Enlarged tonsils 1 month ago	24	
14	9	Congenital syphilis	Acute otitis media 3 years ago	24 Neg.	

Patient	Age	Disease	History of recent acute infection	Dissolution of plasma-clot*
	years			minutes
15	44	Arteriosclerosis	Not significant	20
16	47	Hypertension	Not significant	15
17	64	Hypertension	Not significant	35
18	56	Hypertension	Not significant	12
19	52	Emphysema	Not significant	14
20	62	Nodular goitre	Not significant	12
21	61	Arteriosclerosis	Not significant	25
22	54	Gastric ulcer?	Not significant	25
23	50	Benign prostatic hypertrophy	Not significant	11
24	49	Benign prostatic hypertrophy	Not significant	8
25	61	Benign prostatic hypertrophy	Not significant	7
26	50	Tabes	Not significant	9
27	62	Urinary retention	Not significant	10
28	71	Arteriosclerosis	Not significant	19
29	73	Convalescent heat prostration	Not significant	25
30	68	Hypertension	Not significant	10

* Numbers indicate interval of time between clot formation and clot dissolution.

minutes to 24 hours. All of these patients were in good general health at the time blood was obtained. Although the number of cases is too few to justify conclusions, the frequency of acute upper respiratory infections in children may account for the relatively high proportion of cases with increased resistance.

With the plasma-clot from the blood of patients in the older group, fibrinolytic rate was rapid in each instance. Whether, in older, mildly debilitated individuals, uniform susceptibility is significant, cannot be stated.

By combining the tests, which are recorded in Tables I and II, the group of "normal" persons consists of sixty individuals. The time of clot dissolution with the blood in 75 per cent of these tests is classified as susceptible; 68.3 per cent are highly susceptible. These results are not presented for the purpose of attempting to fix arbitrarily an absolute normal but they serve to disclose the relative rate of streptococcal fibrinolysis among a group of individuals not suffering from acute infection.

Repeated fibrinolytic tests with plasma-clot of blood from patients, before and after recovery from hemolytic streptococcus infections

Twelve patients suffering from various manifestations of acute infection with hemolytic streptococci were used for these observations. In each instance the clinical diagnosis was definite, and hemolytic streptococci were isolated from the patients.

In each case specimens of blood were obtained during the acute stages of disease as well as during convalescence. It has also been possible to follow most of the cases for one to five months after discharge from the hospital. The opportunity, therefore, of repeatedly procuring samples of plasma has served as a means of correlating the clinical course of the infection with changes in the rate of dissolution of the plasma-clot from these patients, and of determining, in addition, the length of time, after recovery, that anti-fibrinolytic principles remain in the blood.

A brief account of the clinical course of each case is given. Individual charts are presented which diagrammatically record the course of the temperature, and the more important signs and symptoms. The time required for dissolution of the plasma-clot from the patients' blood is compared to a test, simultaneously done, with the plasma-clot of a normal blood known to be susceptible.

The cases, which are given in detail with accompanying charts, consist of:

1. *Four cases of erysipelas.* Three recovered and one died.

2. *Three cases of scarlet fever.* All recovered; one developed purulent sinusitis.

3. *Five cases of acute tonsillitis.* All recovered. In two cases convalescence was uneventful; in three others, acute purulent complications occurred.

Patient Br. (See Chart 1). History Number 47,893. White, male, age 38 years. Admitted: February 17, 1933. Disease: Erysipelas of face.

Résumé of clinical course. The patient was admitted on the 3d day of disease. Two days before entry he had become weak, dizzy, alternately chilly and feverish, and noticed that his eyes were swollen. He remained acutely ill and the swelling spread to his forehead and nose.

On admission the temperature was 106.4°. There was a typical erysipelatous lesion involving forehead, eyelids, nose, which spread in butterfly distribution
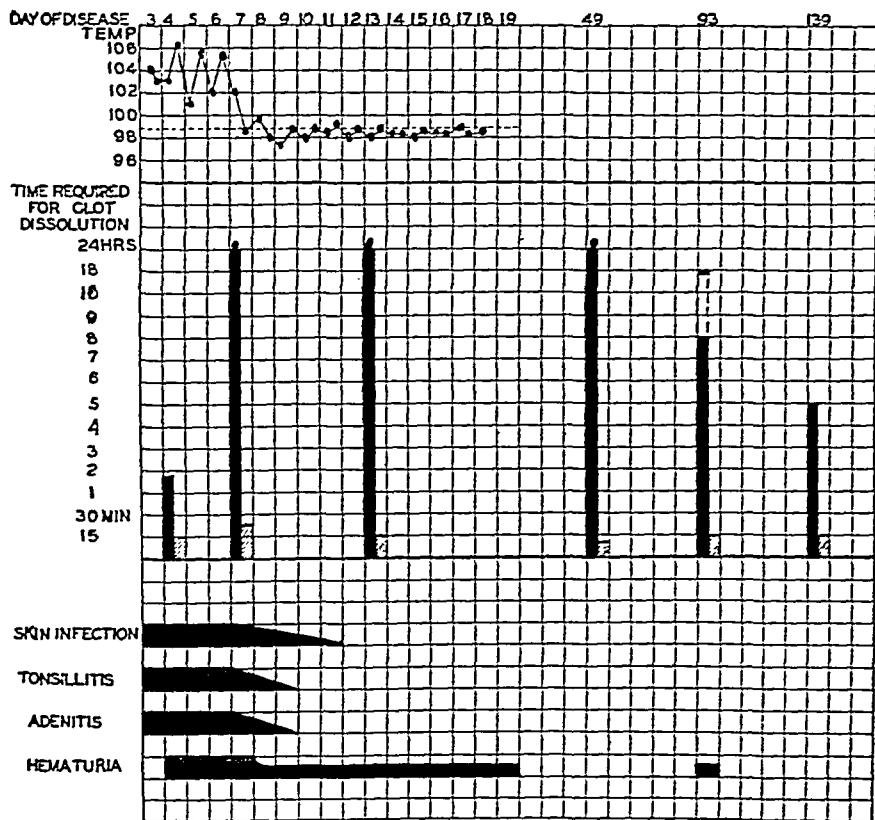
CHART 1. PATIENT BR., MALE, AGE 38 YEARS. DISEASE, ERYSIPELAS

In this and subsequent charts, the upright columns which are recorded at intervals represent fibrinolytic tests with " standard " amounts of plasma and cultures of hemolytic streptococci.

The day on which blood was taken is indicated by the "Day of Disease" at the top of the chart which corresponds to the columns.

The solid black column represents a test with the patient's blood. A flat top to the column indicates the time at which liquefaction was complete; a round dot at the top of the column signifies that the experiment was terminated even though dissolution had not occurred; the section with interrupted lines, which continue above a black column, represents a period of incomplete observation.

The diagonally striated column indicates a control test, simultaneously performed with plasma from a known susceptible.

over cheeks. The tonsils were acutely inflamed, and the cervical glands were enlarged and tender.

Laboratory findings. White blood cells, 13,800 per c.mm. Throat culture: Many colonies of hemolytic streptococci. Blood culture: Sterile. Urine: Trace of albumin, many red blood cells.

Temperature remained high and swinging for four days, during which time the erysipelas spread over the entire forehead and face and involved the lobes of the ears. On the eighth day of disease the temperature fell rather abruptly to normal, the lesion ceased to spread, and symptoms subsided. Recovery was uneventful except for the presence of a few red blood cells in his urine which were present at the time of discharge from the hospital.

Fibrinolytic tests. Samples of blood were obtained on the 3rd day *before* recovery (4th day of disease), on the day of abrupt drop in temperature (7th day of disease), on the 6th day after recovery (13th day of disease), and on 3 different occasions after discharge from the hospital, the last of which was about four and one-half months after recovery.

The fibrin-clot from the plasma obtained during the stage of acute illness was liquefied by active cultures of hemolytic streptococci at a somewhat slower rate than the normal control. The plasma clot from other cases taken during the acute stage of disease has frequently showed a slightly delayed dissolution time. Consideration of this point will be subsequently given. The observation of special interest made with the blood from this case of erysipelas is the sudden appearance on the day of recovery of complete insusceptibility to fibrinolysis. The plasma taken on that day, when clotted in the presence of active culture, remained solid during the 24 hour test period. The potency of the test culture was proved by the rapid dissolution of normal plasma-clot. The resistance to fibrinolysis remained maximum in two subsequent tests on the 7th and 42nd days in convalescence. When tested again about 3 months later, resistance was still high—requiring 8 hours for dissolution—although a decrease from maximum had occurred. In the last test, four and one-half months after recovery, fibrinolysis required 5 hours, indicating a slowly progressive diminution in resistance.

Comment. In this case of erysipelas, maximum resistance to fibrinolysis appeared in the blood coincident with recovery, and has persisted, in gradually decreasing amounts, for four and one-half months.

Patient St. (See Chart 2). History Number 48,419. White, female, age 22 years. Admitted: March 26, 1933. Disease: Erysipelas of the face.

Résumé of clinical course. The acute condition which brought this patient to the hospital was obscure. She had been exposed to mumps and on entry there was a slight degree of tender swelling at the angle of each jaw. There was some soreness of the throat. Whether the swellings were parotid glands or lymph

glands was not clear. After two days the temperature became normal, the condition subsided and patient seemed well. Six days later she had a shaking chill and her temperature suddenly rose to 104° F. She became acutely intoxicated, developed hyperesthesia of forehead and scalp, and the tender swelling at the angle of the right jaw returned. Twenty-four hours later it was evident that she had erysipelas, and the lesion spread rapidly over the forehead, nose, eye-
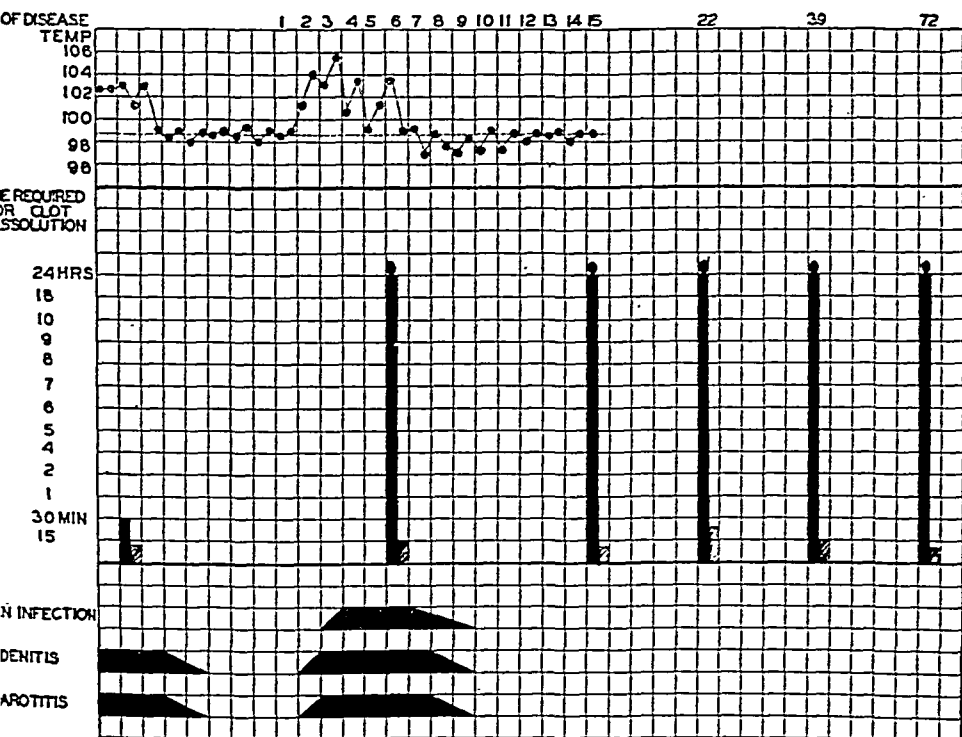
CHART 2. PATIENT ST., FEMALE, AGE 22 YEARS. DISEASE, ERYSIPELAS

lids and face down to the root of the ears. Recovery abruptly occurred five days after sudden onset.

Laboratory findings. White blood cells, 20,000 per c.mm. on admission. 18,000 per c.mm. at time of 2d febrile reaction. Blood cultures: Sterile. Throat culture: Few colonies of hemolytic streptococci present on admission and during erysipelas.

Fibrinolytic test. One specimen of blood was obtained from this patient at the time of the first undiagnosed febrile episode. The rate of fibrin liquefaction was only slightly longer than that of the normal control; 25 minutes as compared to 14 minutes. A second specimen of plasma procured on the day before the temperature suddenly became normal and recovery set in, was resistant to the maximum degree. The blood of this patient has retained maximum resistance at each test, the last of which was performed three months after recovery.

Comment. In this case of erysipelas, maximum anti-fibrinolysis appeared in the blood coincident with recovery from an acute infection of 5 days duration. Insusceptibility to streptococcal fibrinolysis has persisted for three months.

Patient La. (See Chart 3A). History Number 48,508. Male, colored, age 32 years. Admitted: March 30, 1933. Disease: Erysipelas of the face.
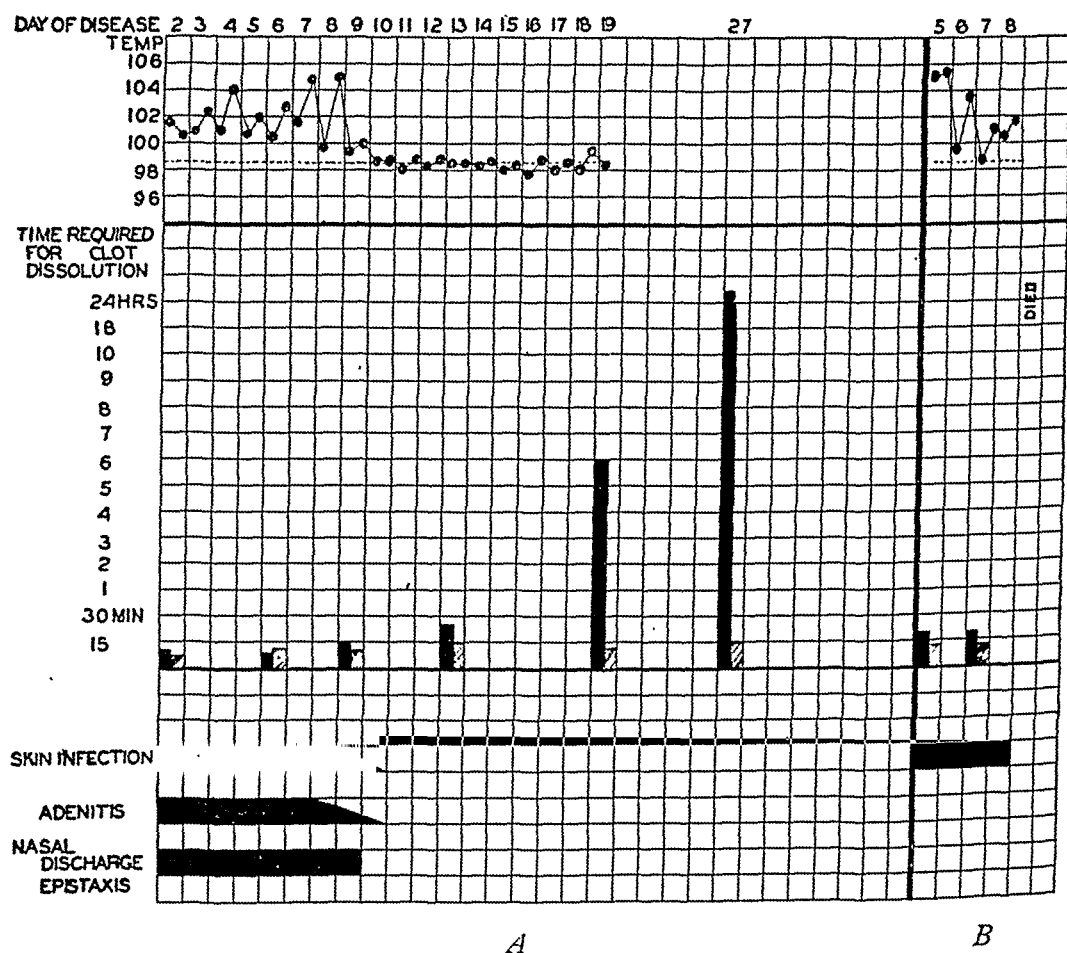
CHART 3A. PATIENT LA., MALE, AGE 32 YEARS. DISEASE, ERYSIPELAS
3B. PATIENT HU., MALE, AGE 72 YEARS, DISEASE, ERYSIPELAS

Résumé of clinical course. The patient was admitted on the second day of disease. Onset was sudden on the day before admission with the development of sore throat, weakness, chills and fever, and headache. The next day his face became swollen, purulent nasal discharge appeared and cervical glands were enlarged and tender. He came to the hospital later on this same day. On admission he was moderately ill, and had the typical lesion of facial erysipelas involving the skin over the bridge of his nose and malar eminences. There was nasal obstruction from the discharge, and tonsils were greatly swollen but not acutely inflamed. Cervical glands were large and tender.

Laboratory findings. White blood cells, 11,720 per c.mm. Blood culture: Sterile. Nasal culture: Many colonies of hemolytic streptococci. Throat culture: Few colonies of hemolytic streptococci.

The erysipelas lesion spread in the usual manner over the entire face, involved ears and mastoid regions, and terminated with a drop in temperature on 9th day after onset. Nose bleeds were frequent during the acute illness. Convalescence was uneventful.

Fibrinolytic tests. In tests made with the first four samples of plasma from this patient, no evidence of resistance to fibrinolysis was demonstrable. The results are of interest since the third specimen was obtained on the day of recovery, and the fourth specimen was procured on the fourth day after recovery. Anti-fibrinolysis first appeared ten days after recovery, and, even then, the clot liquefied in six hours. A sample of blood taken on the 19th day of convalescence remained solid for 24 hours. It is not possible to state when maximum resistance was acquired, since no blood was taken between the 10th and 19th day of convalescence.

Comment. Resistance became demonstrable in this case of erysipelas late in the disease. In the other two patients with erysipelas maximum resistance was present on the day of recovery.

Patient Hu. (See Chart 3B). History Number 48,391. White, male, age 72 years. Admitted: March 23, 1933. Disease: Erysipelas of the face.

Résumé of clinical course. The patient was admitted on the sixth day of disease, which followed a severe blow on the head. He was knocked unconscious and his face was lacerated. The infection began at the wound, and spread over his face and scalp.

On admission he was critically ill and very weak. His face, eyes, and scalp were greatly swollen. He was dyspneic and there was marked arteriosclerosis.

Laboratory findings. White blood cells, 8,500 per c.mm. Blood culture: Sterile. Wound culture: Hemolytic streptococci.

The patient died suddenly three days after admission.

Fibrinolytic tests. Two specimens of blood were obtained from this patient during his acute illness. In both instances the plasma-clot was rapidly liquefied.

Comment. The case is an example of fatal outcome of erysipelas in an individual, age 72 years, whose blood did not contain demonstrable amounts of anti-fibrinolytic substances.

Patient Qu. (See Chart 4). History Number 47,965. White, female, age 20 years. Admitted: February 22, 1933. Disease: Scarlet fever.

Résumé of clinical course. Patient was admitted on second day of disease. Onset was sudden on the day before admission with the development of malaise, sore throat, chill and fever. Rash appeared during the night.

On admission patient was only mildly intoxicated although the throat was fiery red, and typical scarlatinal rash was present over entire body.

Laboratory findings. White blood cells, 16,600 per c.mm. Blood culture: Sterile. Throat culture: Many colonies of hemolytic streptococci.

The patient's symptoms were never severe, her rash faded on the third day, and her temperature, which was never high, became normal on the fifth day. She had vague arthralgia and myalgia. Desquamation began on the fourth day.

Convalescence was normal until the thirteenth day of recovery when she developed tachycardia. The next day her temperature rose to 101.6° F. Cervical glands became moderately enlarged and tender. Four days later the fever disappeared, pulse rate slowed, and the glands subsided. Convalescence was then uneventful.
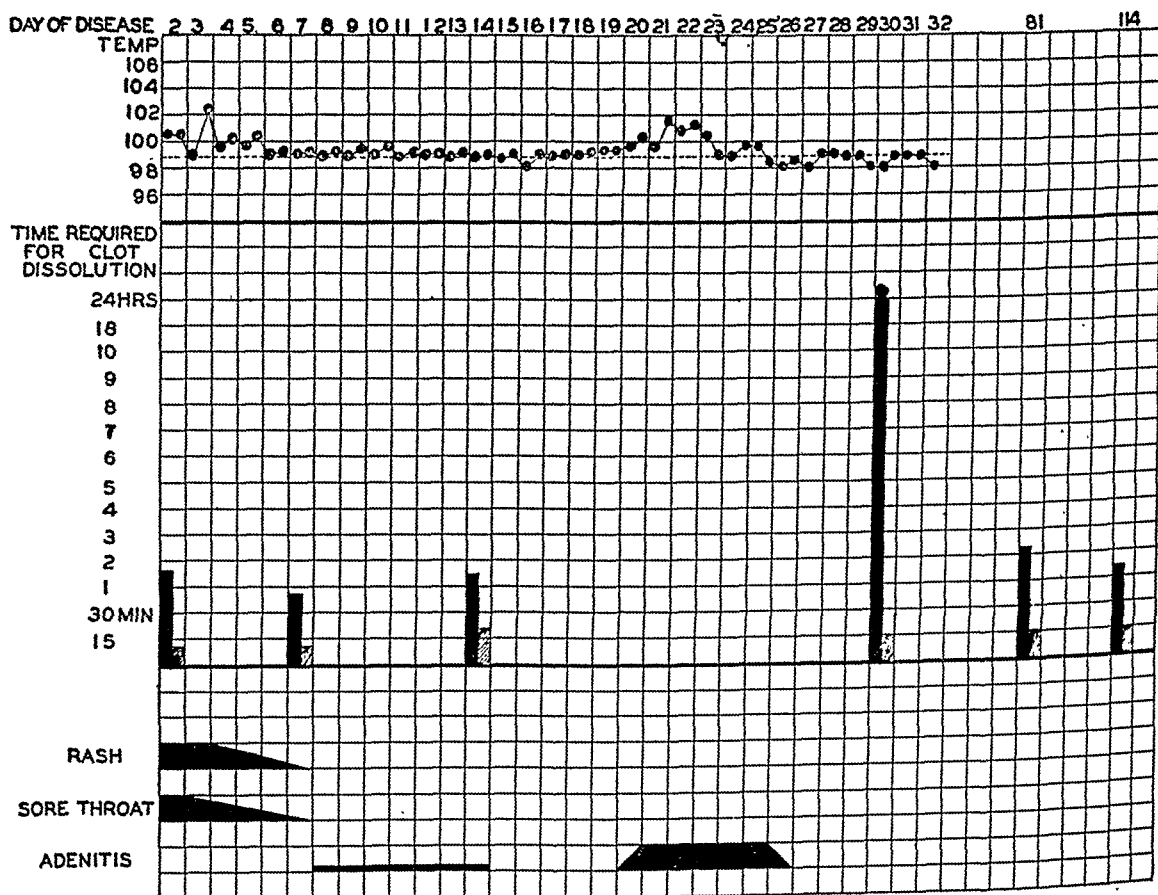
CHART 4. PATIENT QU., FEMALE, AGE 20 YEARS. DISEASE, SCARLET FEVER

Fibrinolytic tests. The fibrin clot from the first sample of blood, taken on the day of admission, required one hour and thirty minutes to dissolve although the normal control liquefied in 12 minutes. With blood procured on the second day of normal temperature, there was only slight difference between the behavior of the patient's plasma and normal plasma. No change in dissolution time was noted in blood taken seven days later. However, maximum resistance was present in the fibrin-clot of blood taken on the 25th day after recovery from scarlatina (5 days after the late febrile episode). With bleedings about 2 and 3 months later, the degree

of resistance had greatly diminished, although the rate of dissolution was longer than the normal control.

Comment. In this case of scarlet fever, maximum resistance was not demonstrable on the second or seventh days of convalescence; but appeared in the sample of blood taken 25 days after recovery, and diminished definitely during the next 3 months.

Patient Os. (See Chart 5). History Number 48,327. White, female, age 20 years. Admitted: March 19, 1933. Disease: Scarlet fever. Treated with scarlatinal antitoxin (2 doses of 6,000 units each, one intramuscularly, and one intravenously).
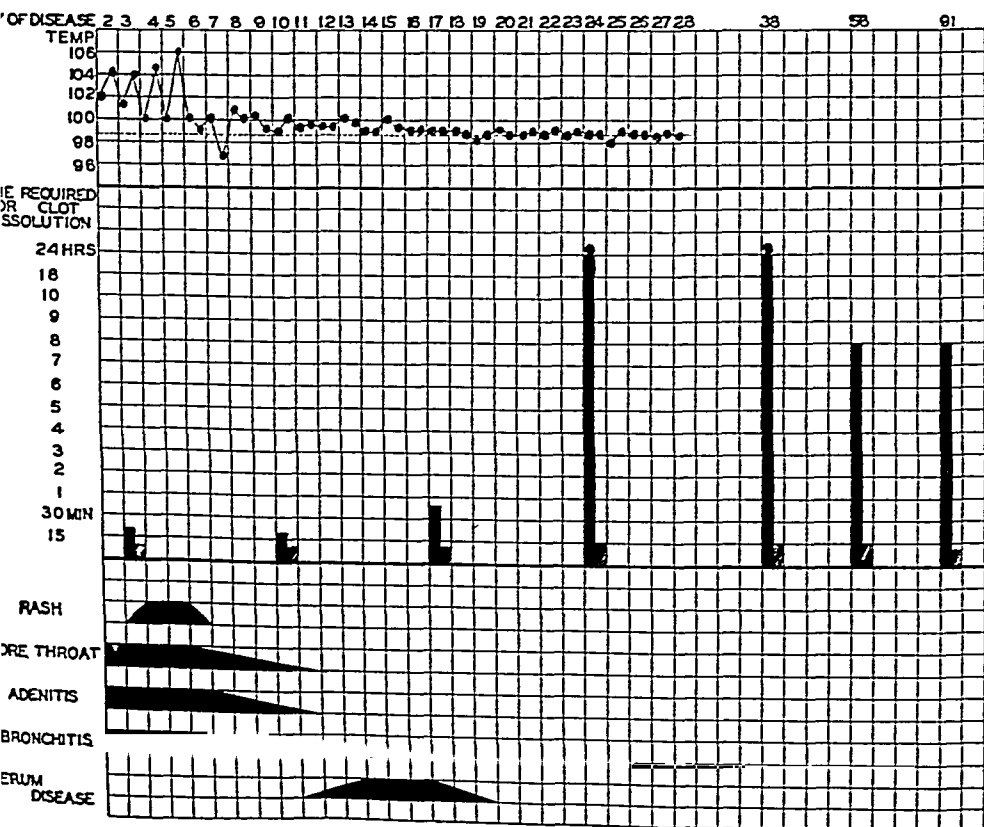
CHART 5. PATIENT OS., FEMALE, AGE 20 YEARS. DISEASE, SCARLET FEVER (TREATED WITH SCARLATINAL ANTITOXIN)

Résumé of clinical course. Patient was admitted on second day of disease. Onset was accompanied by sore throat, malaise, and fever. On admission she appeared acutely intoxicated and her throat, which was acutely inflamed, was very painful. The cervical glands were enlarged and tender. There was a faint erythematous blush in both axillae.

Laboratory findings. White blood cells, 9,800 per c.mm. Blood culture: Sterile. Throat culture: Many colonies of hemolytic streptococci.

Nausea and vomiting developed during the first day, and on the next day a typical scarlatinal rash covered most of the body. She received 6,000 units of antitoxin intramuscularly. On the following day, since no improvement was noticeable, a second injection of 6,000 units of antitoxin was given intravenously. During the next 24 hours, after a chill, the temperature reached a level only slightly above normal, and the rash very quickly faded. Her symptoms were greatly improved.

Eight days later serum sickness developed which lasted about six days. The attack of scarlet fever was not followed by any complications.

Fibrinolytic tests. With the first 3 tests on the blood from this patient, anti-fibrinolytic properties were not demonstrable. The initial bleeding was obtained during acute illness; the other two were taken on the fourth and eleventh days after recovery. Maximum resistance appeared on the 18th day of convalescence, and was maintained to the 32nd day. Two subsequent tests on the 56th and 89th days, respectively, still showed marked resistance, although less than maximum.

Comment. In this case of scarlet fever, as in the preceding one, insusceptibility to fibrinolysis did not become demonstrable until late in convalescence.

Patient Ow. (See Chart 6). History Number 48,425. White, female, age 21 years. Admitted: March 26, 1933. Disease: Scarlet fever. Acute sinusitis. Treated with scarlatinal antitoxin (1 dose intramuscularly of 6,000 units).

Résumé of clinical course. Admitted on first day of disease, moderately ill with fever, sore throat and beginning rash.

Laboratory findings. White blood cells, 11,400 per c.mm. Blood culture: Sterile. Throat culture: Many colonies of hemolytic streptococcus.

During the next 24 hours the rash intensified, and intoxication became marked. Six thousand units of antitoxin were given intramuscularly. The rash faded slowly in the next few days, but the acutely inflamed throat continued to be very painful. On the sixth day she developed signs of acute right maxillary sinusitis. Three days later irrigation of the sinus yielded a moderate amount of thick pus. An abundant growth of *Streptococcus viridans* in pure culture was obtained from the pus. The sinus infection required repeated irrigations and persisted during her stay in the hospital. Other paranasal sinuses were less severely infected. No cultures were obtained from them. Enlarged and tender cervical glands persisted during her illness.

On the 9th day she developed mild serum sickness which disappeared after 5 days.

She was discharged from the hospital with sinus infection still active. Under continual treatment, the infection of sinus has gradually improved. A culture of the discharge from the sinus taken after the patient left the hospital, yielded many colonies of hemolytic streptococci.

Fibrinolytic tests. A sample of this patient's blood taken on the first day of illness, supplied a fibrin-clot which liquefied rapidly. However, the
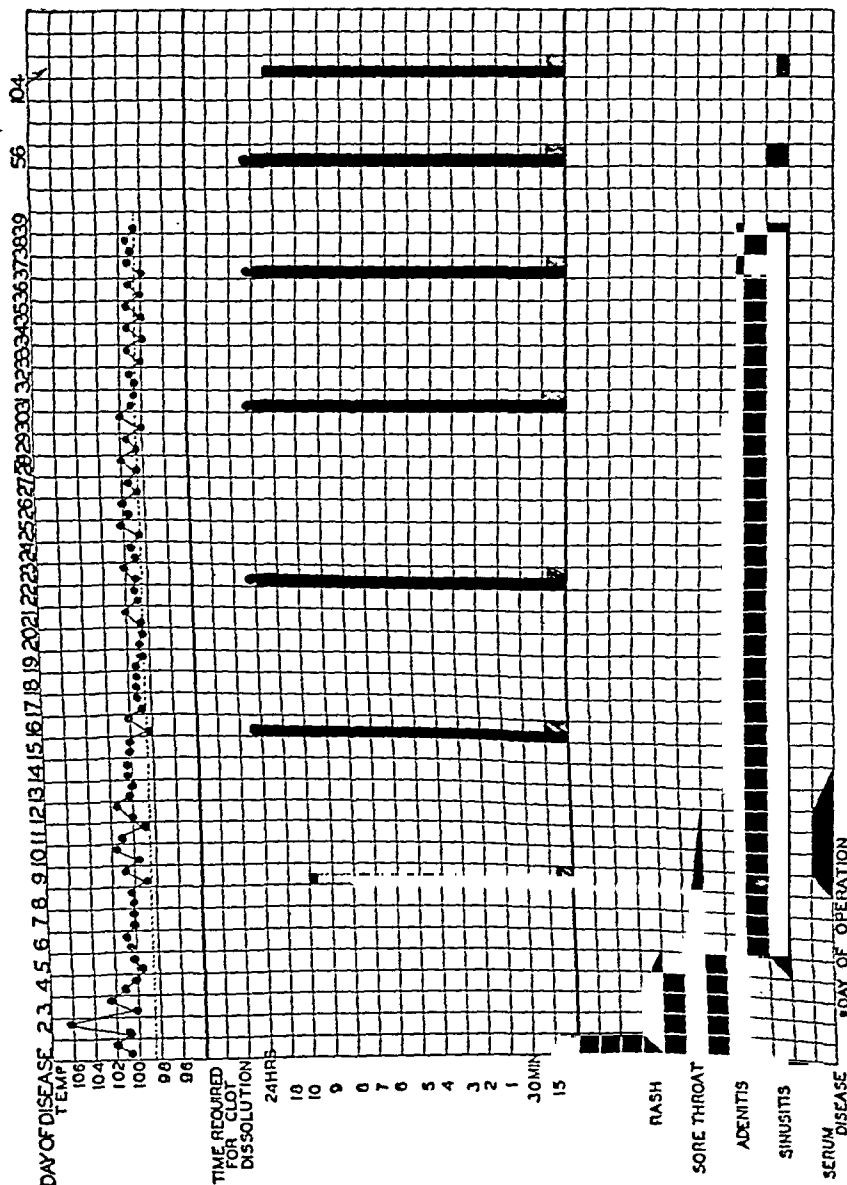
CHART 6. PATIENT OW., FEMALE, AGE 21 YEARS. DISEASE, SCARLET FEVER (TREATED WITH SCARLATINAL ANTITOXIN); ACUTE SINUSITIS

fibrin-clot of a bleeding taken 8 days later—3 days after the first evidence of acute sinusitis, and on the day when thick pus was drained from the sinus—required 8 to 18 hours for liquefaction. A third specimen procured a week later remained solid for 24 hours. This maximum resistance has been maintained up to the time of the last test when liquefaction was complete in 20 hours.

Comment. The development of resistance in this case came earlier than in the other two uncomplicated cases of scarlet fever, and has been maintained to a greater degree for a longer period of time. In this connection it is interesting to note that the sinus infection has required continual treatment and remains as a low grade chronic infection. The appearance of resistance in association with the development of purulent complication is of interest. That the organism in the purulent discharge from one sinus was *Streptococcus viridans* is also of interest, since throat cultures yielded hemolytic streptococci. Cultures from other less severely infected paranasal sinuses were not obtained. A subsequent sinus culture was positive for hemolytic streptococci.

Patient Ba. (Chart 7). History Number 48,074. White, female, 23 years. Admitted: March 1, 1933. Disease: Acute tonsillitis (Hemolytic streptococcus).
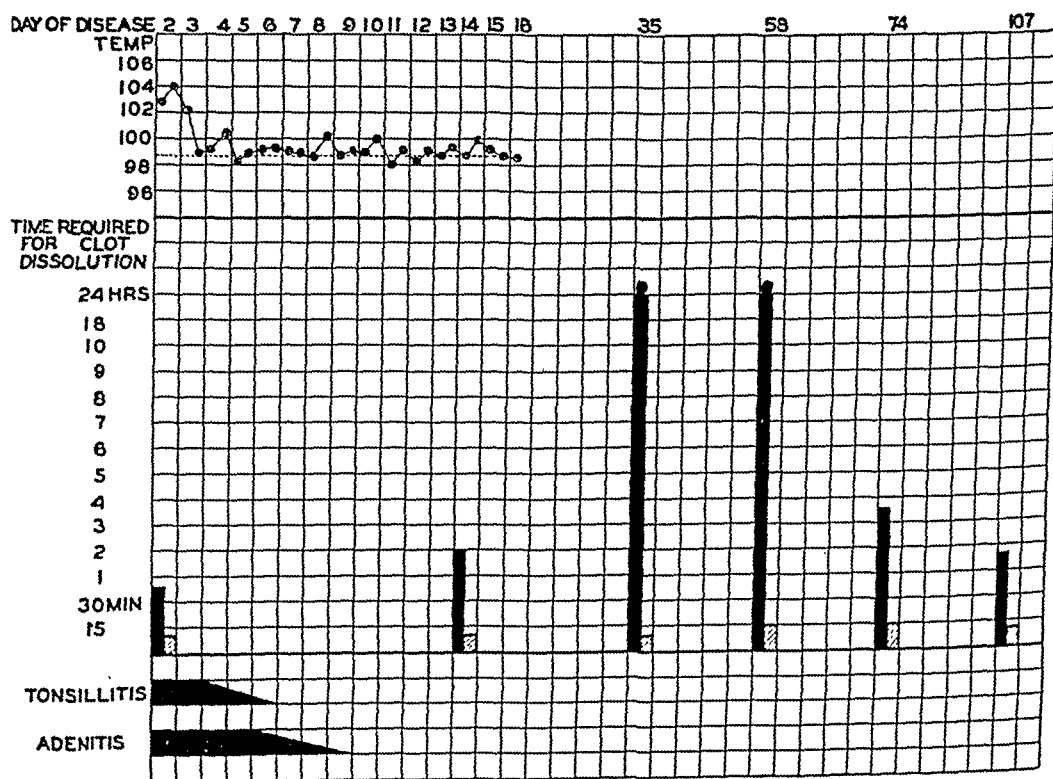
CHART 7. PATIENT BA., FEMALE, AGE 23 YEARS. DISEASE, ACUTE TONSILLITIS

Résumé of clinical course. Patient was admitted to the hospital on the second day after the sudden development of acute sore throat.

Laboratory findings. White blood cells, 16,800 per c.mm. Throat culture: Many colonies of hemolytic streptococci.

The throat was very acutely inflamed, and the tonsils were spotted with yellowish-white exudate. Swallowing was painful. General intoxication was marked. Cervical glands were large and tender.

Temperature became normal on the third day, and symptoms subsided rapidly. There were no complications.

Fibrinolytic tests. Repeated tests with the plasma-clot from this case of uncomplicated acute hemolytic streptococcus tonsillitis show that, although slight delay in fibrinolysis was evident in a sample of blood taken 8 days in convalescence, maximum resistance was first demonstrable on the 35th day after recovery. Insusceptibility to dissolution endured for 3 weeks, but subsequent tests showed a rapid diminution of resistance, which 4 months after the acute illness was only slightly manifest.

Comment. In this case of acute tonsillitis, as in the two patients with uncomplicated scarlet fever, resistance was demonstrable late in convalescence. Since no observations were made between the 13th and 39th day, the exact time of development of resistant properties is undetermined. The duration of acute illness was only four days.

Patient Hi. (Chart 8). History Number 43,979. White, female, age 26 years. Admitted: March 12, 1933. Disease: Acute tonsillitis (Hemolytic streptococcus).

Résumé of clinical course. The patient, admitted on the second day after abrupt onset, had a mild attack of acute tonsillitis. Tonsils were inflamed and edematous. Cervical glands palpable and slightly tender.

Laboratory findings. White blood cells, 8,750 per c.mm. Throat culture: Many colonies of hemolytic streptococci.

After two days in bed, recovery was complete. There were no complications.

Fibrinolytic tests. In this patient, a test with the plasma-clot procured on the 7th day of convalescence was not significantly different from the first test. With blood taken on the 22nd day of convalescence fibrinolysis required 8 hours. Subsequent observations, on the 43rd, 59th and 109th days, showed a persistence of resistance at a lower level.

Comment. This patient had a mild attack of uncomplicated acute tonsillitis of short duration. Resistance appeared late in convalescence and was never maximum.

Patient Fu. (Chart 9). History Number 48,423. White, female, age 21 years. Admitted: March 25, 1933. Disease: Acute pharyngitis (Hemolytic streptococcus). Acute maxillary sinusitis.

Résumé of clinical course. Patient was admitted to the hospital on the second day of disease, complaining of sort throat and headache. She was
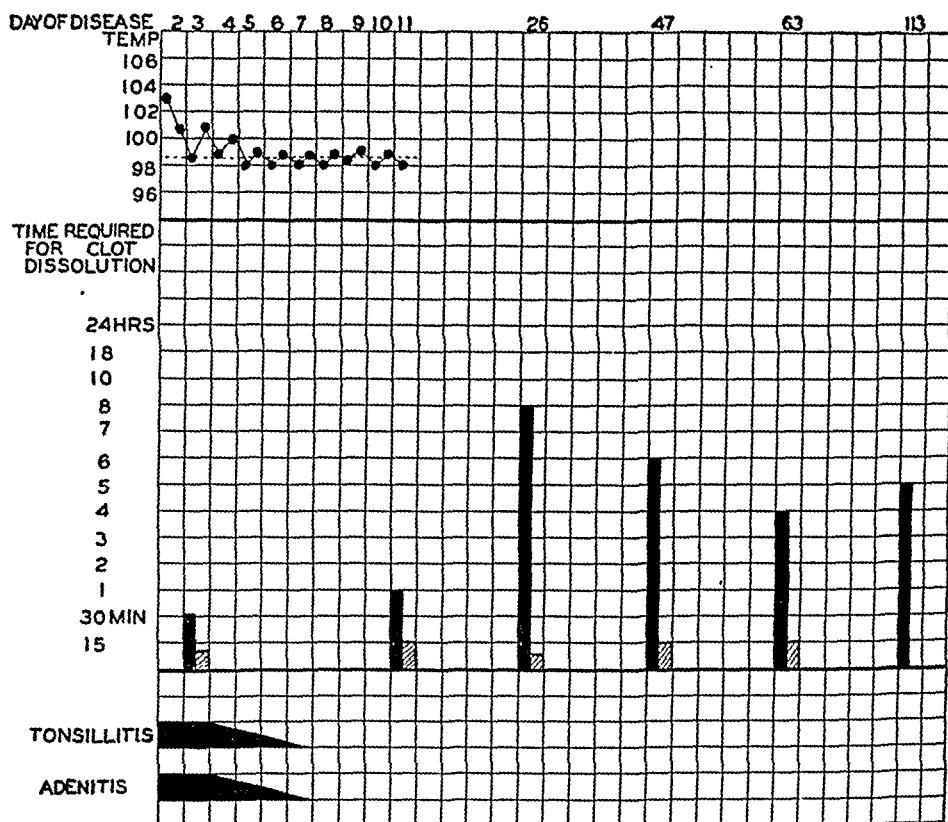
CHART 8. PATIENT HI., FEMALE, AGE 26 YEARS. DISEASE, ACUTE
TONSILLITIS

moderately ill; her throat was acutely inflamed, and there was tenderness over the left antrum.

Laboratory findings. White blood cells, 14,500 per c.mm. Throat culture: Few colonies of hemolytic streptococci.

Two days after admission the pain in the left antrum became very severe, and after two days of ineffectual local treatment, the sinus was irrigated. Bloody mucoid exudate was discharged. Culture of pus yielded only pneumococcus (Group IV).

Convalescence was rapid and uneventful. The sinus infection subsided after drainage and did not return.

Fibrinolytic tests. The first sample of plasma from this patient was definitely resistant. It was obtained on the 3rd day of acute pharyngitis which was complicated by acute sinusitis. Nine days later a fibrinolytic test showed an additional increase in resistance. Three weeks later resistance had decreased but was still marked. One and one-half months and three months after the acute illness, fibrin-clot from the patient's plasma was completely susceptible.

Comment. Resistance was manifest early in this patient. Symptoms of sinusitis appeared very quickly after the beginning of the throat infection. The sinusitis healed readily, and resistance disappeared more quickly than in the other patients. The isolation of pneumococcus from the in-

* Day of operation.
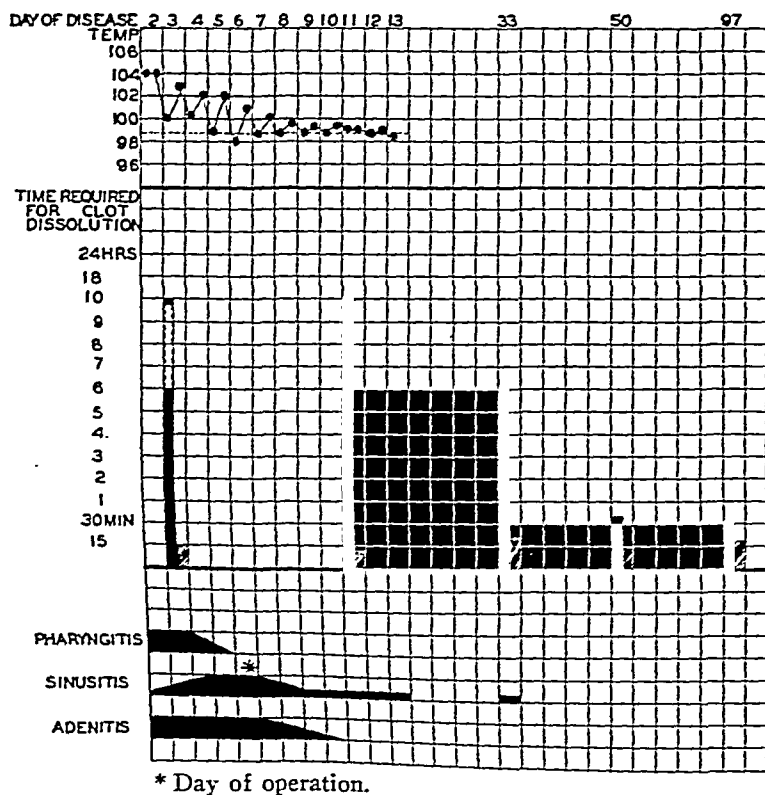
CHART 9. PATIENT FU., FEMALE, AGE 21 YEARS. DISEASE, ACUTE PHARYNGITIS; ACUTE SINUSITIS

fected antrum is of interest, in view of the fact that hemolytic streptococci were present in the throat.

Patient Ch. (Chart 10). History Number 48,745. White, female, age 21 years. Admitted: April 13, 1933. Disease: Acute maxillary sinusitis, bilateral (Hemolytic streptococcus). Acute otitis media, bilateral (Hemolytic streptococcus). Acute pharyngitis (mild) (Hemolytic streptococcus).

Résumé of clinical course. The patient was admitted to the hospital on the 5th day of an acute febrile upper respiratory tract infection with involvement of both maxillary sinuses on the third day of disease, and acute right otitis media on the fourth day of disease. The illness began with pharyngitis which was never severe, and was not prominent on admission. Exquisite tenderness and pain over the sinuses and in the ears constituted the chief complaint. Right myringotomy was performed on the first day in the hospital with the evacuation of thin serosanguineous exudate. Later in the same day, left ear became painful, and the ear drum was incised; thin pus was obtained. Both antra, which were acutely infected, were also irrigated.

Cultures of pus from both ears and both antra yielded abundant bacterial growth, which was predominently hemolytic streptococcus.

Laboratory findings. White blood cells, 17,200 per c.mm.

Although adequate discharge of pus relieved the severity of symptoms, fever persisted for 12 days. When the patient left the hospital, the ears and sinuses had apparently completely healed.
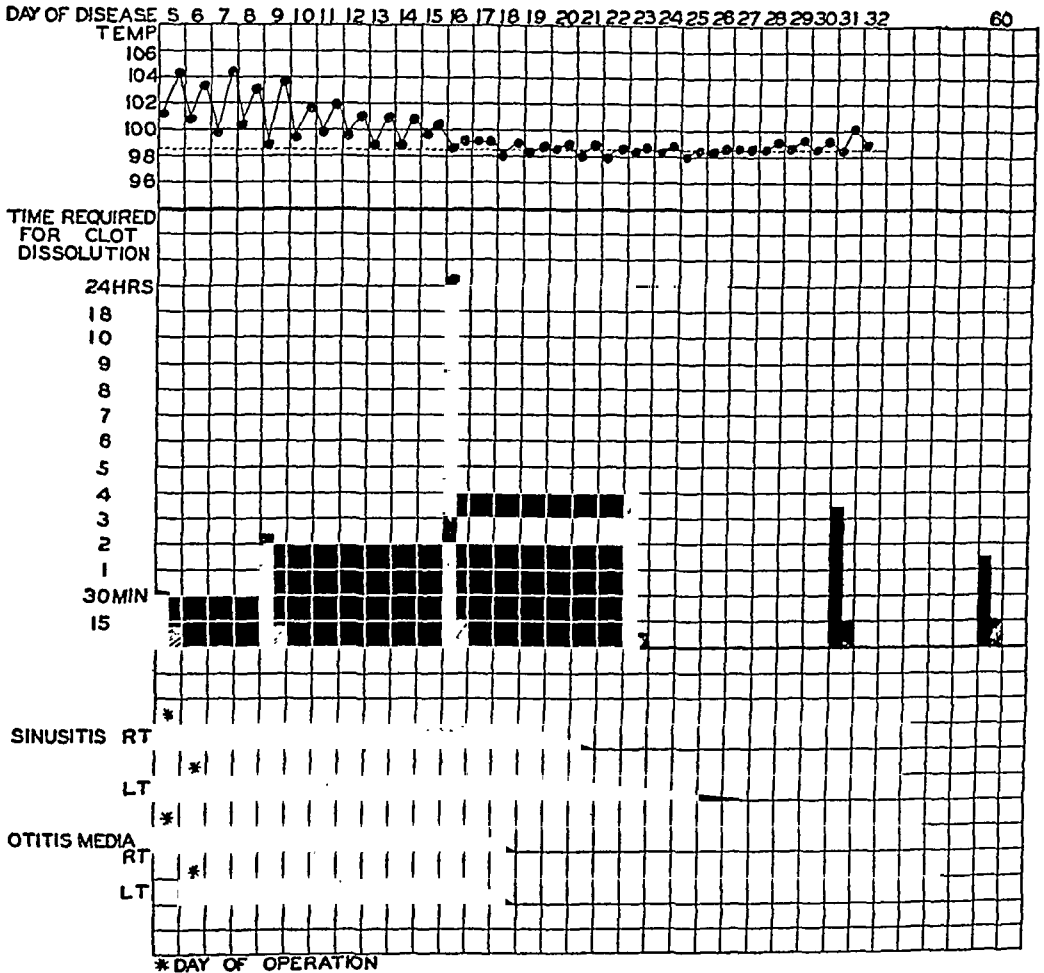
CHART 10. PATIENT CH., FEMALE, AGE 21 YEARS. DISEASE, ACUTE SINUSITIS; ACUTE OTITIS MEDIA

Fibrinolytic tests. Tests with the blood of this patient, who had multiple purulent inflammations, reveal that, early in the active disease, fibrinolysis proceeded rapidly. Four days later, delay in liquefaction was definite but not marked. No additional tests were taken until the 16th day, at which time anti-fibrinolysis was maximum. From this point on, subsequent tests showed progressive diminution in delayed liquefaction.

Comment. The appearance of maximum resistance to fibrinolysis occurred in the blood of this patient with purulent complications of upper respiratory infection at a time when the condition was subsiding. Complete insusceptibility did not seem to appear quite as abruptly as in the other

cases with suppuration; but it was demonstrable earlier than in the blood of patients without complications. Recovery from infection was complete in about 3 weeks and marked resistance was not maintained for a long time.

Patient V.H. (Chart 11). History Number 40,215. White, female, age 24 years. Admitted: February 27, 1933. Disease: Acute otitis media (Hemolytic streptococcus).

* Day of operation.
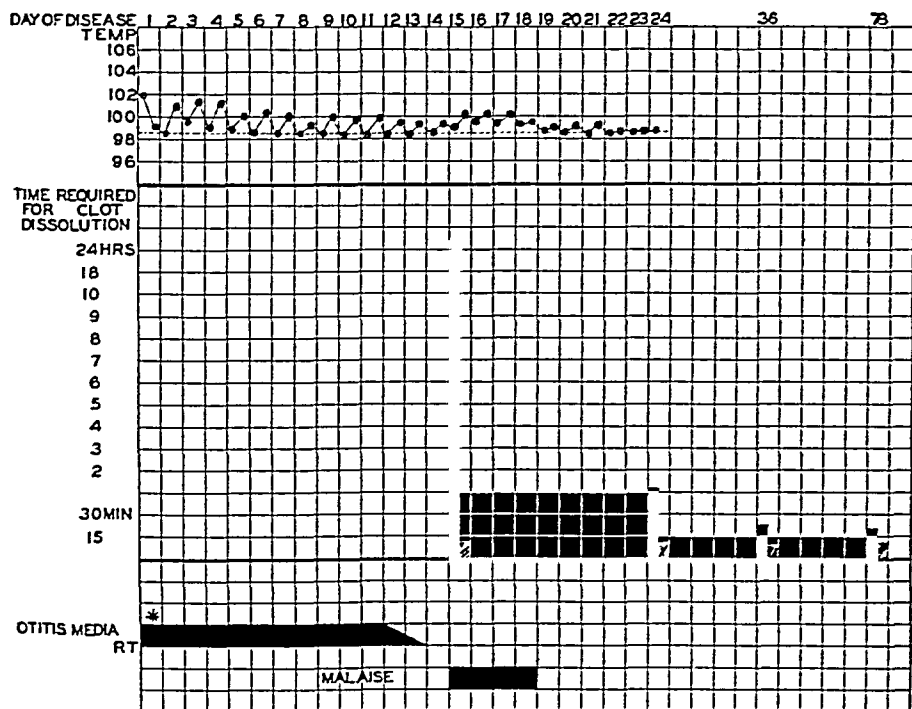
CHART 11. PATIENT VH., FEMALE, AGE 24 YEARS. DISEASE, ACUTE OTITIS MEDIA

Résumé of clinical course. After two days of a mild nasopharyngitis, patient developed suddenly acute severe pain in right ear. She was admitted to the hospital this same day and myringotomy was performed at once. Thin serosanguineous fluid was evacuated.

Laboratory findings. White blood cells, 5,000 per c.mm. Later 13,500 per c.mm. Ear culture: Heavy growth of pure culture of hemolytic streptococci. Throat culture: Heavy growth of pure culture of hemolytic streptococci.

Adequate drainage was difficult to maintain, and three subsequent myringotomis were necessary. Pain in the ear and temperature gradually subsided in about a week. On the 13th day temperature became slightly elevated again, and she had general malaise and aching. After 3 or 4 days, the symptoms subsided, and recovery was uneventful. At the time of discharge from the hospital the ear had stopped draining. There was slight impairment to hearing.

Fibrinolytic tests. Unfortunately, tests were not done early in this patient's illness. It is, therefore, impossible to state whether the patient's plasma-clot was susceptible to dissolution at the beginning. The first test, which was made with a sample of blood taken on the 15th day showed maximum resistance. Three subsequent tests during the 2 months after discharge from the hospital showed that resistance had disappeared completely.

Comment. The results of fibrinolytic tests with the blood of this patient exemplifies the presence of maximal resistance in a person with suppurative hemolytic streptococcus infection, and the subsequent rapid disappearance of anti-fibrinolytic properties from the blood following complete recovery.

In all the cases of acute streptococcus disease, with which Charts 1 to 11 are concerned, it may be seen that convalescence is, in every instance, attended by the presence in the patients' plasma-clot of maximum or very marked resistance to the fibrinolytic property of hemolytic streptococci. That fibrin from the same individuals, obtained before and after recovery, changed from susceptible to maximally resistant in a short period of time evidences the fact that infection with hemolytic streptococci stimulates the production of anti-fibrinolytic substances. Resistance was equally marked in the blood of patients convalescent from erysipelas, scarlet fever, and acute tonsillitis, with or without complications.

The duration of anti-fibrinolytic resistance has varied but seems to be influenced by the nature and length of time that active infection persists. Individual reactivity, also, undoubtedly participates in the degree and extent of this mechanism as in other immunological responses.

The number of cases of each type of infection is too small to attach undue significance to the time in the course of the disease at which resistance became marked. The following résumé serves, however, as a summary.

Two cases recovered from erysipelas developed maximal resistance on the day of recovery. (Charts 1 and 2.)

One case recovered from erysipelas developed marked resistance 7 days after recovery, which was found to be maximum 8 days later. (Chart 3.)

One fatal case of erysipelas failed to develop resistance. (Chart 3.)

Two cases recovered from uncomplicated scarlet fever developed maximal resistance on the 17th and 24th days respectively. (Charts 4 and 5.)

One case of scarlet fever, complicated by purulent sinusitis on the 4th day, developed resistance on the 6th day. (Chart 6.)

Two cases recovered from acute tonsillitis without complications were maximally resistant in tests done on the 26th and 35th days of disease. (Charts 7 and 8.)

Three cases recovered from acute tonsillitis with suppurative complications exhibited marked or maximum resistance in tests done on the 3d,

15th, and 16th days respectively after the appearance of purulent infections.   (Charts 9, 10 and 11.)

In the 8 cases of upper respiratory streptococcal infection, the development of resistance appeared later in the 4 uncomplicated cases than it did in the 4 patients with purulent complications.   Whether or not a purulent inflammatory process hastens this immune response cannot yet be finally stated.   However, the delayed anti-fibrinolytic response in some instances, and the immediate response in others, forms a striking part of the results of this investigation.

Repeated fibrinolytic tests with plasma-clot of blood from patients, before and after recovery from other infections

In order to determine whether or not resistance to the fibrinolytic activity of hemolytic streptococci is specifically induced in streptococcus infections, a series of repeated control tests have been performed with plasma from cases of other acute diseases.

The results with eleven of these patients is contained in Charts 12 to 15 inclusive.   The infections from which the patients suffered are as follows:

Five cases of pneumococcus pneumonia; 1 case of typhoid fever; 1 case of gonococcal arthritis; 1 case of active pulmonary tuberculosis; 1 case of malaria (Therapy for general paresis); 1 case of acute gangrenous appendicitis (Colon bacillus); 1 case of abscess of muscle (gram positive, anaerobic bacillus).

In each of these patients the clinical and bacteriological diagnosis was definite; hemolytic streptococcus infection was ruled out.   Except for one case, a detailed account of the clinical course is unnecessary.

In Chart 12, the results of repeated fibrinolytic tests with the blood of four cases of pneumococcus pneumonia are given.

Case Th.   (History Number 49,201.   Admitted: May 11, 1933) had lobar pneumonia due to Type VII pneumococcus.   He recovered uneventfully.

Case Ja.   (History Number 49,035.   Admitted: April 30, 1933) had lobar pneumonia due to Type IV pneumococcus.   He recovered without complications.

Case Na.   (History Number 46,954.   Admitted: December 18, 1932) had Type III pneumococcus pneumonia, with uncomplicated recovery.

Case Ha.   (History Number 49,572.   Admitted: May 31, 1933) had an overwhelming Type I pneumococcus septicemia at the time of full term pregnancy, and died on the 6th day of disease in spite of anti-pneumococcus serum therapy.

From Chart 12 it may be seen that clot liquefaction occurred rapidly with each sample of blood taken either before or after recovery.   In Case Th., the last test was made with plasma-clot obtained on the 40th day after recovery.   Case Na. was repeatedly tested at intervals for 33 days.   Case Ja. was not available after the 19th day; only one specimen of blood was obtained from the fatal case, Case Ha.
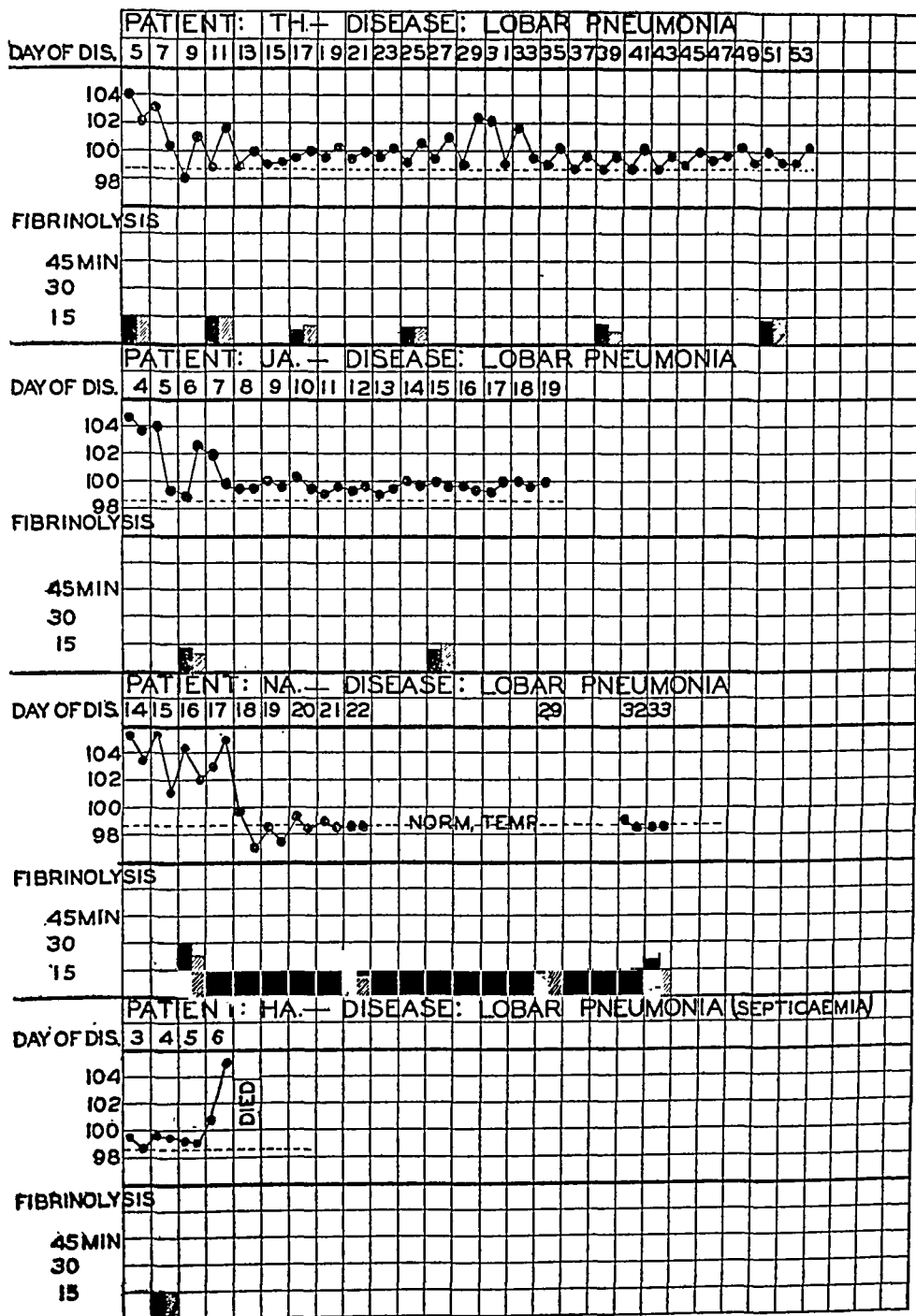
CHART 12. PATIENTS TH., JA., NA., AND HA.

The fifth case of pneumonia, Case Pa., presented results which are unique and have not been noted in any other individuals. Although no explanation can be offered at the present time, the change in reactivity of the patient's blood was sufficiently striking to justify presentation.

Case Pa. (See Chart 13). (History Number 49,363. Admitted: May 25, 1933.) The patient was admitted to the hospital seriously ill with Type I pneumococcus pneumonia and septicemia. He was treated vigorously with concentrated anti-pneumococcus serum and recovered without complications. It is interesting to note that 2 months before admission he had trouble with his right ear which was followed by paralysis of some of the right cranial nerves. Because of the fact that he was known to have syphilis, the neurological condition was thought to be recurrent neural syphilis in an individual who had received inadequate treatment. That his spinal fluid was normal, however, threw doubt on the correctness of the suggested diagnosis.
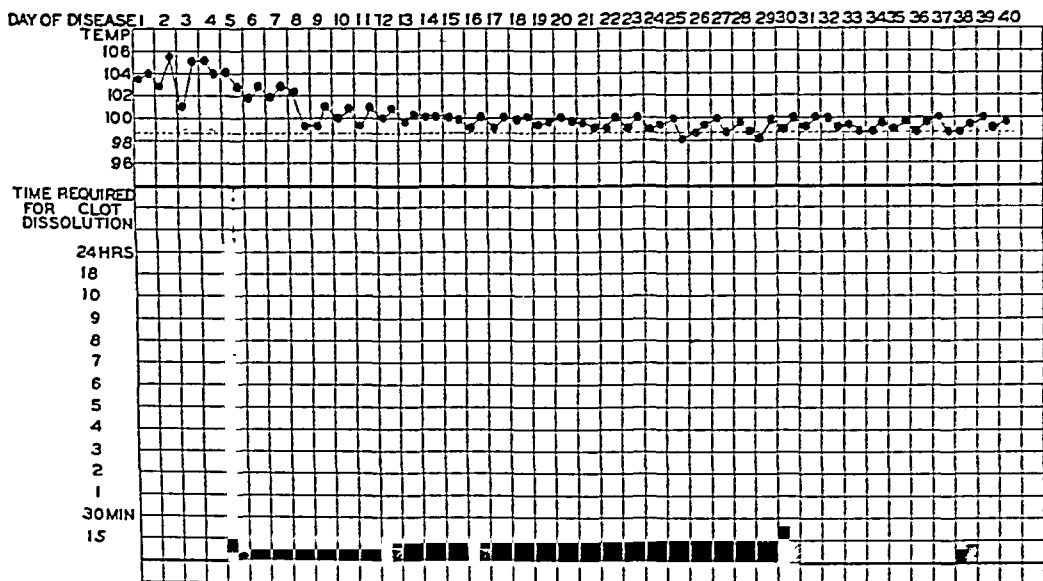
CHART 13. PATIENT PA. DISEASE, LOBAR PNEUMONIA (TYPE I SEPTICEMIA) (SERUM THERAPY)

From the patient's chart it may be seen that the fibrin clot from the sample of blood taken on admission was totally resistant to fibrinolysis (Chart 13). Seven days later, the anti-fibrinolytic properties had entirely disappeared. Furthermore, in all bleedings for the next 4 weeks, no return of resistance was demonstrable.

The presence of maximum resistance in this patient's blood at the time of early severe illness has not been encountered in any of 8 other cases of pneumonia, whose blood was tested during the acute disease. That the initial resistance might have resulted from the infection of the ear two months previously is a possibility. The abrupt disappearance of resistance is inexplicable at the present time, and one can only speculate as to the effect which large amounts of anti-pneumococcus serum might have on the inhibiting properties of the blood. The last bleeding in this patient was taken 31 days after recovery.

In Chart 14 the results with three other cases of different types of infection are presented.

Case Sw. (History Number 48,821. Admitted: April 18, 1933) had acute gonococcal arthritis with multiple joint involvement. Gonococci were present in purulent exudate obtained from the joints.

Case Ca. (History Number 48,929. Admitted: April 24, 1933) had active pulmonary tuberculosis. The sputum contained many tubercle bacilli.

Case An. (History Number 36,328. Admitted: May 14, 1933) had typhoid fever. Typhoid bacilli were present in the blood stream and also in the stools.

The plasma-clot from the blood of each of these three patients was liquefied in every test by active cultures in less than one hour. By following the cases with repeated bleedings for at least 30 days, no change in dissolution time was noted. That the plasma-clot from patients acutely ill liquefies at a *slightly slower rate than that of the susceptible normal control*, has been repeatedly observed. However, the rate of dissolution occurring with the blood of the three cases presented above belongs to the group designated susceptible.

Three additional cases of infection, with whose blood repeated fibrinolytic tests have been performed, are recorded in Chart 15.

Case Sc. (History Number 49,333. Admitted: May 19, 1933) had general paresis and was admitted to the hospital for therapeutic malarial infection. The intermittent fever indicates the time of active malaria, which was terminated by quinine therapy. Blood was obtained before, during, and after malarial inoculation.

Case Bo. (History Number 49,751. Admitted: June 13, 1933) had an acute cellulitis of the leg, which, after several days of palliative treatment, was incised. A gram positive anaerobic bacillus was isolated from the pus. No hemolytic streptococci were present. Blood was taken before and after operation.

Case Cr. (See Chart.) (History Number 49,284. Admitted: May 13, 1933) had acute appendicitis. At operation the appendix was found to be gangrenous, and there was some localized peritonitis. Drainage was carried out for several days and recovery was uneventful. Colon bacilli were obtained from the pus. The first sample of blood was obtained after operation.

From Chart 15 it may be seen that resistance is absent from the blood of these three patients and that repeated tests did not reveal any change in the rate of dissolution during the period of observation.

The results of fibrinolytic tests with the plasma-clot of blood from the cases of acute illness just described indicate that resistance to streptococcal fibrinolysis is not a general reaction to infection. In ten of the selected cases with whose blood repeated tests were made, the rapid rate of fibrinolysis did not change either early or late after recovery. The unique behavior of the case of Type I pneumonia, treated with specific anti-serum, whose blood abruptly lost resistance is a single exception. Even in this
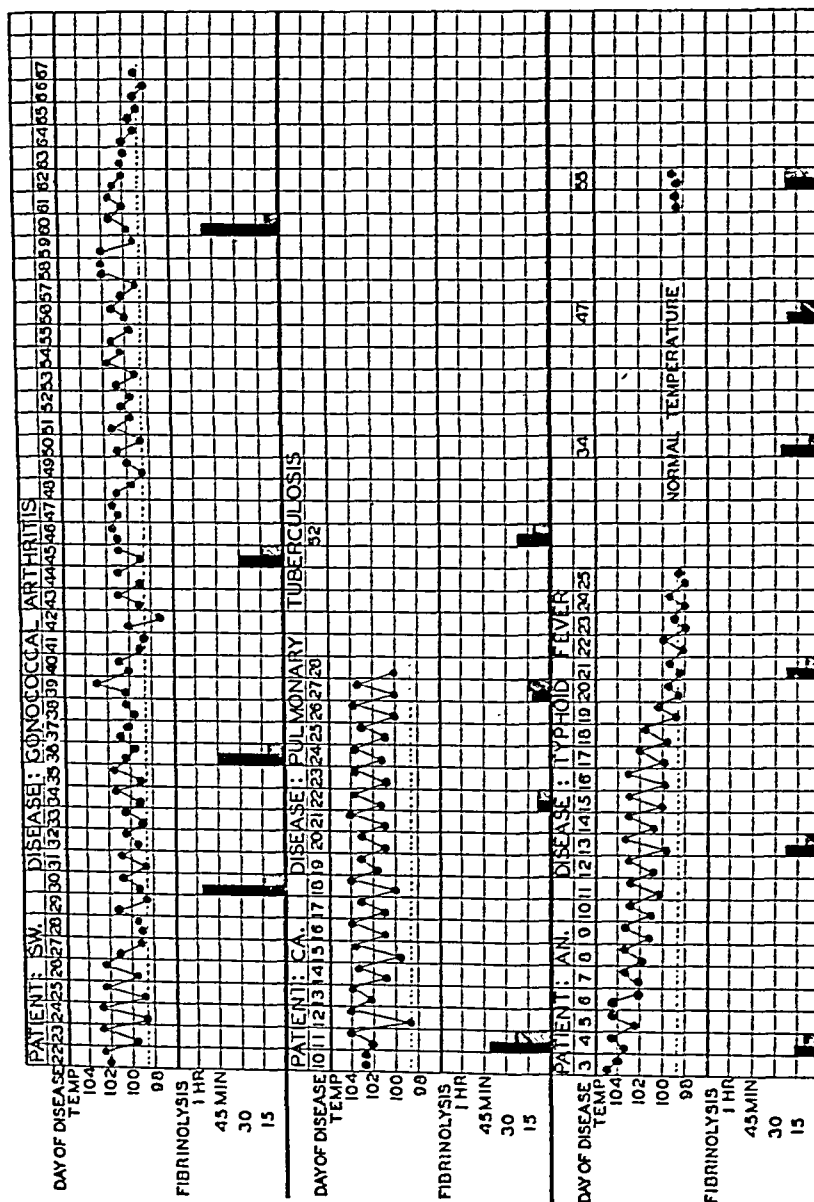
CHART 14.   PATIENTS SW., CA., AND AN.
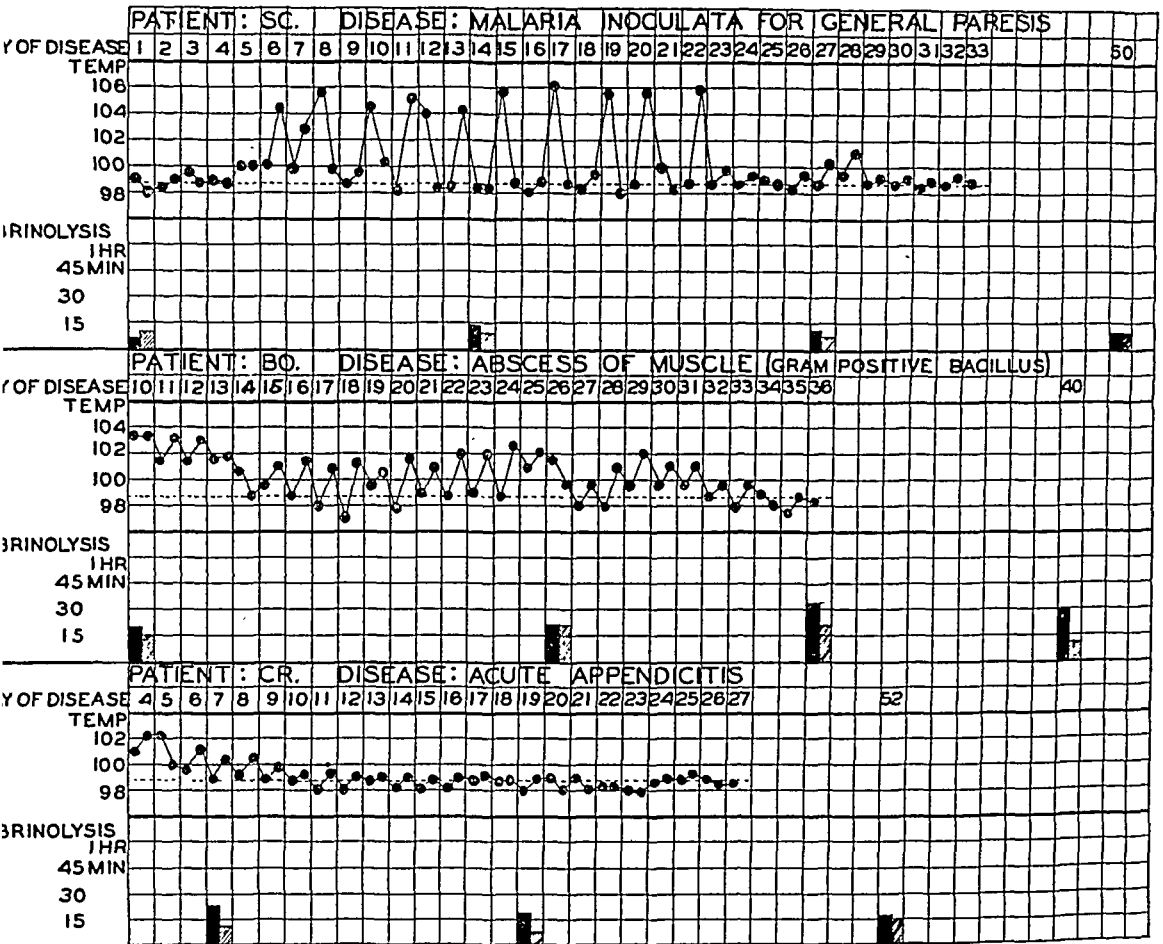
CHART 15.   PATIENTS SC., BO., AND CR.

case, the changes were the reverse of the course in patients with hemolytic streptococcus infections.

Repeated fibrinolytic tests with the plasma-clot from normal healthy adults

The observations recorded in Chart 16 were made with samples of blood obtained from normal adults, who were separately tested over a period of several months.   The purpose of this study was to determine what variations in fibrinolytic rate occurred in an individual in whom the factor of disease was not present.   Five normal persons were, therefore, bled at weekly or monthly intervals for 4 months.   During this time they had no acute illnesses except mild coryza in the early spring.

The plasma-clot from the blood of individuals T., O., and G. were highly susceptible to fibrinolysis on every test.   Among these three the greatest variation was with the blood of O., which on one test required 10 minutes for dissolution and on another required 40 minutes.

The blood of individuals S. and F. possessed, at the beginning, definite resistance to fibrinolysis, the plasma-clot of S.'s blood requiring 4 hours

to liquefy, and that of F.'s requiring 3 hours. The activity of the test culture was proved by suitable control. The delayed rate of dissolution present in the fibrin from the initial bleeding remained essentially the same in each of 5 specimens of blood obtained at intervals for about 4 months.
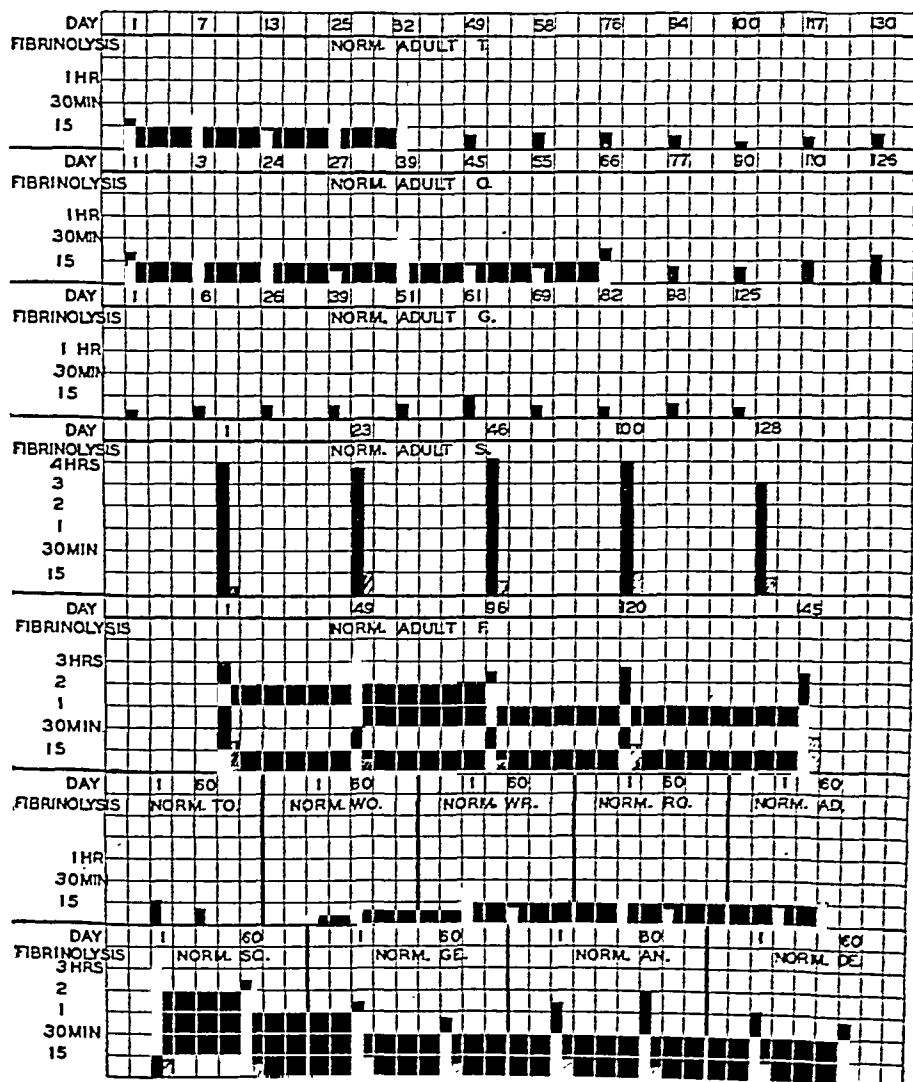
CHART 16. NORMAL ADULTS

Neither of these individuals gave a history of acute infections during the past few years.

In fibrinolytic tests with the blood of these five normal adults, therefore, it may be seen that, at least for as long as 4 months, the individual reactivity is not subject to significant variation.

The uniformity of the individual reactivity is also evident in the tests which are recorded in the two bottom rows of Chart 16. These observations consist of 2 separate fibrinolytic tests with blood from each of 9 healthy adults. The samples of blood were taken 2 months apart. Individuals To., Wo., Wr., Ro. and Ad., were highly susceptible in each test, whereas the others, Sc., Ge., An. and De., possessesd definite resistance which was maintained with only slight variation.

The presence of some degree of anti-fibrinolytic resistance in the blood of a few healthy adults may be interpreted as an enduring partial response to some earlier infection or as an individual property natural to the person's blood. From all the charts it may be noted that there is frequently some discrepancy between the patient's blood and the normal control. Since it was impossible to know the behavior of the patient's blood before the disease began, the result of tests with the first bleeding cannot be accurately evaluated. It would seem, however, to be due either to the individuals own natural reactivity or to an immediate response to infection. One factor of infection, which undoubtedly contributes to the results, is the quantitative increase in blood fibrinogen which is known to occur early in many acute infections. The answer to this phase of the problem must await further study.

DISCUSSION

The observations, which comprise this report, are part of an investigation of the fibrinolytic properties of hemolytic streptococci. It has been of interest to attempt to determine whether or not this biological property of the organisms may be significant either as a factor in virulence or as an agent capable of evoking an immunological response. A study of the relationship of bacterial fibrinolysis to tissue invasion is now in progress but is not complete. The observations, which are given in detail in this article, concern the immunological phase of the problem.

The results, indicate that, following acute infection with hemolytic streptococci, the blood of the patient acquires a new property by means of which the plasma-clot becomes highly resistant to dissolution by active cultures of hemolytic streptococci.

The resistance, stimulated by acute streptococcus infection, was demonstrable in the blood of patients convalescent from erysipelas, scarlet fever, and acute tonsillitis. The time in the course of convalescence, at which maximum resistance to fibrinolysis was demonstrable, varied from an immediate response to a delay of several weeks. Some of the cases developed suppurative complications. Among these there was a tendency for resistance to appear more quickly than in the uncomplicated cases. This result suggests that the presence of pus may hasten the appearance of anti-fibrinolytic substances. It will be of interest, therefore, to investigate, in

a larger series of cases, the relationship of suppuration to anti-fibrinolytic resistance, and to observe whether the character of the exudate changes in accordance with the ability to withstand the lytic power of the infecting agent.

In contrast to the anti-fibrinolytic response which follows streptococcus infection, the blood from patients with other acute illnesses did not contain significant amounts of anti-fibrinolytic substances effective against the active principle of streptococci, nor was convalescence in this control group of non-streptococcal infections attended by any change in rate of fibrinolysis.

Observations of clot dissolution with blood from healthy adults and from cases of low grade chronic disorders reveal, in about 75 per cent of the tests, high susceptibility to fibrinolysis. The varying degrees of resistance exhibited by the blood of the remaining 25 per cent cannot yet be interpreted. It seems probable either that it represents evidence of a previous streptococcus infection or indicates " natural immunity." Repeated tests on healthy adults show that, over a period of several months, the individual fibrinolytic rate does not significantly change; individuals, whose blood manifested susceptibility, remained so, and the plasma-clot of others, which was moderately resistant, maintained the same delayed rate of liquefaction. The constancy of the reaction in normal persons adds additional significance to the changes which occur following acute streptococcus disease.

The results, therefore, of repeated fibrinolytic tests with blood from cases of acute hemolytic streptococcus infections, and of comparable observations with blood from other types of acute infection, as well as from healthy individuals, have been interpreted as signifying that insusceptibility to fibrinolysis is specifically induced; that the fibrinolysin of hemolytic streptococci, in the body, evokes a definite response directed against the lytic action of the bacteria. The exact nature of anti-fibrinolytic substances, however, has not yet been determined.

Although the observations recorded in this article do not concern the fibrinolytic activity of hemolytic streptococci from the standpoint of bacterial pathogenicity, the fact that patients respond to infection by acquiring a means of combating the agent of fibrinolysis, might be considered as indirect evidence that this bacterial property is of some significance. It is, therefore, justifiable to comment briefly upon the possible rôle which a biological substance having the capacity to dissolve fibrin with great rapidity, might play in relation to bacterial virulence.

If the deposition of fibrin, which occurs early in the process of inflammation, is part of the attempt to wall off the infecting agent it becomes evident that the invasion of tissues might be influenced by bacterial fibrinolysin. Micro-organisms, under the circumstances, which are capable of breaking down the fibrin wall by dissolution would—barring other im-

munological processes such as agglutination or phagocytosis—be free to spread through the tissues.

When the clinical course of certain acute fulminating hemolytic streptococcus infections is considered in the light of the highly active fibrinolytic properties of cultures of this organism, a causal relationship suggests itself. For example, the thin character of the exudative reactions, especially notable in acute streptococcus infections of serous surfaces, may find a rational interpretation on the basis of the liquefying property of these organisms. The walling-off process of recovery, which often consists of a barrier of fibrin deposition, may represent the anti-fibrinolytic immune reaction exemplified by the results contained in this article. The study of the relation of streptococcal fibrinolysis to virulence is proceeding along the lines indicated by the interpretation just mentioned.

CONCLUSIONS

Under the experimental conditions described, the plasma-clot from the blood of patients convalescent from acute hemolytic streptococcus infections was found to be highly resistant to fibrinolytic principles contained in active cultures of hemolytic streptococci. By repeated tests with samples of blood, obtained before and after recovery, it was possible to demonstrate, in the same individual, the development of anti-fibrinolysis.

In comparable tests with the blood from a limited number of other types of bacterial infection, the recovery of the patients was not followed by the appearance of anti-fibrinolytic substances in the blood.

The plasma-clot of the blood from 75 per cent of sixty so-called " normal " persons was highly susceptible to fibrinolysis.

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ANTIBODY RESPONSE TO INFECTIONS WITH TYPE III AND THE RELATED TYPE VIII PNEUMOCOCCUS [1]

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Artificial immunity to the Type III pneumococcus varies with different animal species and differs from that obtainable with Types I and II (1). The antibody response, in man, to lobar pneumonia due to Type III is less constant and of lower grade than that following infection with the latter types. The Type VIII pneumococcus (2), which is immunologically related to but not identical with Type III (3), has been found frequently in association with human disease (4). In the present communication are presented the results of tests for pneumococcus antibodies in patients with infections associated with Type III and with Type VIII pneumococci.

EXPERIMENTAL

Subjects, materials and methods

The sera of 71 patients with infections associated with Type III or Type VIII pneumococci were studied. Patients with lobar pneumonia, bronchopneumonia or other infections without pneumonia were included. The pneumococcus type was usually obtained from a culture of the heart's blood of a mouse inoculated with sputum. All cultures were agglutinated both macroscopically and microscopically in Type III and Type VIII antisera, progressive dilutions of the sera being used where cross-agglutination was encountered. In many instances subcultures of colonies from the surface of blood agar plate cultures were used for typing. Blood cultures were made by inoculating, at the bedside, 5 to 10 cc. of blood into beef infusion broth at pH 7.8 and pneumococci thus obtained were similarly typed. Typing sera for Types I to XXXII (5) were obtained from the Laboratories of the New York City Department of Health through the kindness of Miss Georgia Cooper and Dr. William H. Park. Additional sera for Types I, II and III were furnished by Dr. Benjamin White of the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Public Health and by Dr. Augustus B. Wadsworth of the Laboratories of the New York State Department of Health.

The materials and methods used in testing for agglutinins and mouse protective antibodies were similar to those employed in other studies (6). Further antigens were obtained from single colony cultures of strains encountered during this study. The tests for cross-agglutination of strains of pneumococci

were made with fresh, live, fully grown (10 to 14 hours), plain broth, single-colony cultures incubated with serial dilutions of typing sera for 1 hour at 56° C. and the readings made after overnight icebox storage. Only floccular agglutinations were considered positive.

Absorption experiments were carried out with freshly prepared, heat-killed, saline suspensions of pneumococci, the packed sediment of 50 to 150 cc. of a fully grown culture being used for each cubic centimeter of serum. The mixture was incubated at 37° C. for 2 hours, with frequent shaking, then stored in the icebox overnight and the cleared supernatant used for agglutination and protection tests.

RESULTS

Agglutination of Types III and VIII strains in anti-pneumococcus horse sera

Tests for cross-agglutination were carried out with 6 Type III and 21 Type VIII strains of pneumococci recently isolated from the sputum, blood or lungs of pneumonia patients. One Type VIII and 3 Type III horse antisera from different laboratories were used. Two of the Type III antisera agglutinated homologous strains in dilutions up to 1:40 or 1:80, and the third up to 1:160 or 1:640. One of the first 2 sera failed to agglutinate 5 Type VIII strains and agglutinated the rest only when undiluted or in dilutions up to 1:4; the other agglutinated all Type VIII strains, usually in dilutions up to 1:20 or 1:40. The third Type III serum failed to agglutinate most Type VIII strains. The Type VIII antiserum agglutinated homologous strains in dilutions up to 1:80 or 1:160. This serum failed to agglutinate 4 Type III strains and agglutinated two others only in 1:2 dilutions. Microscopic agglutinations carried out in each instance with 1:5 dilution of the different antisera, showed corresponding differences in the occurrence and character of the agglutination observed.

The " typing " sera were thus found to vary considerably in the degree to which they cross-agglutinated strains of pneumococci of the related type. This was not dependent on the titers of homologous agglutinins.

Antibody response to infections associated with Types III and VIII pneumococci

The results of the agglutination and protection tests with both Types III and VIII pneumococci in the sera of patients with Type III infections are shown in Table I. Except as indicated in this table, each of these patients had lobar pneumonia clinically and by x-ray, and Type III pneumococci were obtained from the sputum on one or more occasions. The blood cultures were sterile in all the recovered patients and in one-half of the fatal patients. Similar data for the Type VIII patients are given in Table II. The sputum of each of these patients had Type VIII pneumococci on one or more examinations. The results of the blood cultures are indicated in each instance. The data in Tables I and II are summarized in Table III.

TABLE I

Antibody response to infections associated with Type III pneumococci *

Case number	Patient	Age (years)	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. III	Agglutinins Pn. VIII	Mouse protection Pn. III	Mouse protection Pn. VIII	Remarks
1	J. H.	22	Lysis	10	8	0	0	0	0	
					22	8	0	10	0	
2	E. C.	44	Lysis	22	23	0	0	0	0	
					32	32	0	10^2	0	
3	A. S.	49	Lysis	7	3	0	0	10^3	0	Bronchopneumonia, bronchial asthma. Readmitted for asthmatic attack after 3 months. Agglutinins for Pn. VII (1 : 4) in last serum
					10	64	0	10^3	0	
					67	4	0	10^2	0	
					74	4	0	—	—	
					124	4	0	10^2	—	
4	M. I.	52	Crisis	8	8	0	0	0	10^2	
					19	64	0	10^3	10^4	
5	A. G.	31	Crisis	4	2	0	0	0	0	
					5	4	0	—	—	
					8	2	0	0	0	
					13	2	0–2	10^2	10^3	
					19	2	0–2	10^3	10^3	
6	D. VanF.	39	Lysis, Recrudescence	23? 26–34	25	8	8	10^3	—	Pn. III in sputum 22nd day, Pn. VII on 30th day. Agglutinins and protection for Pn. VII absent 31st day and present on the 40th day. (Agglutinins 1 : 4, protection 10^6)
					31	8	4–8	10^4	10	
					40	4	2	10^4	10	
7	T. C.	54	Lysis	9	12	4	0	—	0	
					33	4	0	10^4	0	
8	E. E.	26	Crisis	8	7	16	0	—	—	
					8	32	0	—	—	
					10	32	0	10^3	0	
9	W. L.	37	Lysis	7	10	4	0	10^3	0	Readmitted 82nd day with acute upper respiratory infection. Lungs clear. Only Pn. X and Pn. XVII recovered from sputum on repeated examination on 2nd entry. No agglutinins for the latter types in any of the sera.
					19	4	0	10^3	0	
					83	0	0	0	10^3	
					94	0	0	0	10^4	
10	F. O'B.	44	Lysis	14	14	8	0	—	—	Bronchopneumonia
					22	4	0	—	—	

TABLE I—(continued)

Case number	Patient	Age	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. III	Agglutinins Pn. VIII	Mouse protection Pn. III	Mouse protection Pn. VIII	Remarks
11	D. R.	18 (years)	Crisis	6	6	4	0	10^3	10	Postoperative lobar pneumonia
12	S. S.	72	Crisis	6	11	2	0	10^2	0	
13	F. G.	65	Crisis	5	13	8	0	10^3	0	
14	T. A.	42	Crisis	9	7	8	0	10^4	0	Agglutinins (1 : 8) and protection for Pn. V (10^5) in this serum
15	E. H.	42	Crisis	10	6	0	0	—	—	
16	J. S.	52	Lysis	14	20	0	4	0	10^3	Bronchopneumonia. No pneumococci recovered from sputum 12th day, Pn. III obtained on 14th day
					12	0	2–4	0	10^5	
17	M. W.	69	Lysis	10	18	0	0	0	0	
					8	0	0	0	0	
18	M. K.	41	Crisis	12	11	0	0	0	0	
					8	0	0	0	0	
					15	0	16	0	10^3	
					19	0	4	0	10^2	
19	F. L.	48	Crisis	5	5	0	0	0	0	Bronchopneumonia and pulmonary tuberculosis
20	M. D.	62	Lysis	12	10	0	0	0	0	
					18	0	0	0	0	
					30	0	0	0	0	
21	P. Ci.	36	Pseudo-crisis	11 / 20	6	0	0	0	0	Recrudescence 15th to 20th day. Pn. III from sputum 10th day. Only Pn. V in sputum 12th and 15th day. Agglutinins (to 1 : 32) and protection (to 10^4) for Pn. V
					11	0	0	0	0	
					18	0	0	0	0	
					24	0	0	0	0	
					31	0	0	0	0	
22	W. W.	58	Lysis	8	12	0	0	0	0	Postoperative bronchopneumonia
					17	0	0	0	0	
					24	0	0	0	0	
					31	0	0	0	0	
23	R. W.	50	Crisis	5	20	0	0	0	0	
					9	0	0	0	0	
24	J. O'B.	36	Lysis	8	8	0	0	0	0	Pn. III and Pn. VIII (no Pn. II) in sputum on 3rd day. Pn. II (no Pn. III or Pn. VIII) in sputum on 6th day. Agglutinins (to 1 : 8) and protection for Pn. II (10^4) found 10th day and later
					10	0	0	0	0	
					12	0	0	0	0	
					23	0	0	0	0	

TABLE I—(continued)

No.		Age	Result							Remarks
25	F. Co.	39	Died	9	7	0	0	—	—	Pn. III from blood culture on 7th day
26	M. L.	50	Died	10	9	0	0	—	0	Blood culture sterile 6th day, showed Pn. III on 7th day
27	D. McC.	49	Died	10	7	0	0	0	10^2	Blood culture sterile 4th and 5th days, showed Pn. III on 9th day. Agglutinins for Pn. II (1 : 2) and Pn. V (1 : 4) without protection on 7th day, none on 9th day
28	S. T.	60	Died	9	9	16	2	10^3	—	Bronchopneumonia complicating carcinoma of lung. Blood cultures: 7th day, negative; 9th day Pn. III; at autopsy, negative
29	M. G.	57	Died	9	7	32	0	10^3	0	Blood culture sterile on 7th day, bronchopneumonia
30	S. J.	54	Died	22	19	0	0	0	0	Bronchopneumonia. Blood culture sterile on 19th day
31	H. P.	24	Lysis	3	7	4	0	10^2	0	"Grippe." No pneumonia. Pn. III and Pn. VIII in sputum
32	C. W.	33	Lysis	3	8	0	0	—	—	"Grippe." No pneumonia
33	F. Ce.	38	Lysis	7	11	0	0	0	10^3	Influenza. Lungs clear
34	M. D.	30	—	—	9	0	0	0	0	Pulmonary tuberculosis, febrile, positive sputum
35	M. H.	41	Lysis?	6	8†	0	0	0	0	Fractured ribs, bloody sputum with Pn. III, 8 days later had fever for 6 days. No evidence of pneumonia.

TABLE II—(continued)

Case number	Patient	Age years	Blood culture Result	Blood culture Day	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. III	Agglutinins Pn. VIII	Mouse protection Pn. III	Mouse protection Pn. VIII	Remarks
47	C. McC.	32	Negative	3	Lysis	12	4	0	0	0	0	
			Pn. VIII	4			9	0	0	—	—	
			Negative	7			16	0	0	0	10^4	
			Negative	12			23	0	2	—	—	
							25	0	4–8			
48	E. F.	36	Negative	3	Lysis	4	3	0	0	0	0	
							10	0	4–16	10	10^5	
							17	0	4–8	0	10^5	
							22	0	8	—	—	
49	J. A.	43	Pn. VIII	2	Crisis	6	5	0	0	0	0	Diffuse bronchopneumonia
			Pn. VIII	5			8	0	2	10	10^3	
							19	0	2	0	10^4	
50	C. L.	45	Negative	8	Crisis	8	12	2	16–32	10^2	—	Bronchopneumonia
							16	4	32	10^4	10^4	
51	J. McLe.	42	Negative	11	Lysis	12	22	0	8–16	0	10^5	
52	S. H.	58	Negative	6	Lysis	8	9	2	4–8	10^2	10^3	
							14	2	8–16	10^3	10^4	
							20	0	2–4	10^3	10^5	
53	E. S.	18	—	—	Crisis	6	12	4	4	10^4	10^5	
							15	4	4–8	10^4	10^4	
54	F. H.	52	Negative	—	—	—	—	16	0–2	10^5	10^3	

TABLE II—(continued)

Case number	Patient	Age	Blood culture Result	Blood culture Day	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. III	Agglutinins Pn. VIII	Mouse protection Pn. III	Mouse protection Pn. VIII	Remarks
		years										
55	J. C.	40	—	—	Crisis	5	7 11 11	0 0 0	0 0 0	0 | 0	0 | 10	Bronchopneumonia
56	W. B.	60	Pn. VIII	4	Lysis	9	4 8 18	0 0 0	0 2 0	0 | 0	0 | 0	
57	F. DeB.	48	Negative Negative	5 7	Crisis Recurrence	3 9	7 13	0 0	0 0	| 0	| 0	Extended after pseudocrisis
58	R. J.	42	Pn. VIII	6	Lysis	12	7 9 15 27	0 0 0 0	0 0 0 0	0 | 0 0	0 | 0 0	Pn. XVIII recovered from 1 of 4 sputa. No other pneumococci found. Thrombophlebitis 13-27th day
59	W. J.	40	Negative	14	Crisis	16	13 18	0 0	0 0	0 0	0 0	
60	W. T.	45	Negative	6	Lysis	8	11 16 22	0 0 0	0 0 0	0 0 0	0 0 0	
61	H. T.	34	Negative	5	Crisis	5	5	0	0	0	0	
24	J. O'B.	36	Negative	8	Lysis	8	5	(see Table I)				Also has Pn. III in sputum (see previous table)

TABLE II—(continued)

Case number	Patient	Age (years)	Blood culture Result	Day	Termination Mode	Day	Day of serum	Agglutinins Pn. III	Agglutinins Pn. VIII	Mouse protection Pn. III	Mouse protection Pn. VIII	Remarks
62	E. Ha.	59	Negative / Negative	5 / 9	Died	11	5 / 9	0 / 0	0 / 32–64	0 / 0	0 / 10^5	Autopsy: Bronchopneumonia. Cultures: Heart's blood = *Strep. hem.* Lungs = *Strep. hem.* and *Staph. aureus*
63	J. R.	36	Negative	5	Died	5	5	0	0	0	10^4	Bronchopneumonia
64	W. W.	40	Negative / Negative	3 / 5	Died	9	9	0	0	—	—	
65	G. T.	35	Pn. VIII	3	Died	4	4	4	0	0	0	
66	F. H.	52	Pn. VIII	24	Died	30	30	29	0	0	10^2	
67	J. P.	48	—	—	Died	25	25	10	0	0	10^4	Acute laryngitis; no pneumonia. Also had Pn. III in sputum
68	F. D.	38	—	—	Crisis	8	8	11	4	0	0	"Grippe," no pneumonia
31	H. P.	24	Negative	—	Lysis	4	3	7	0	0	0	"Grippe," no pneumonia
69	W. D.	25	—	—	Crisis	—	4	6	0	0	0	Postoperative fever; no pneumonia. Pn. VIII in one of 4 throat cultures (Pn. X, XXIII and XXXI in others). Agglutinins for each of these types absent
70	C. S.	50	—	—	Crisis	—	5	13	0	0	0	
71	A. Y.	55	—	—	Improved	—	—	—	3 times	0	0	Pulmonary infarct (Pn. III in sputum 3 months previously)

TABLE III

Summary of Tables I and II: Immunity and cross-immunity resulting from infections associated with Types III and VIII pneumococci

Patients' type	Number tested	Only homologous positive*	Only heterologous positive*	Both positive	Both negative	Agglutination			Mouse protection		
						Number tested	Agglutinins demonstrated		Number tested	Protection demonstrated	
							Pn. III	Pn. VIII		Pn. III	Pn. VIII
Pneumonias recovered — III	24	8	2	5	9†	24	13	4	21	11	7
VIII	22	6	0	9	7†	22	5	14	22	9	13
Pneumonias fatal — III	6	2	1	0	3	6	2	1	5	2	1
VIII	6	2	1	0	3	6	0	1	4	1	2
Infections without pneumonia — III	10	2†	1	0	7	10	1	0	8	2	1
VIII	5	1	1†	0	3	5	1	0	5	1	1

* Homologous and heterologous refer only to Types III and VIII tests in relation to the type obtained from the patient.

† Cases 24 and 31 had both Type III and Type VIII pneumococci and are listed twice.

It will be seen from these tables that the serum of one-half of the Type III and two-thirds of the Type VIII patients with pneumonia who recovered and one-third of those who died had agglutinins and protective antibodies for the homologous type pneumococcus late in the disease, or during convalescence. Sera taken early in the disease showed no such antibodies. Cross-agglutination and cross-protection between the Types III and VIII were frequent in patients who had either of these types. With some exceptions, the patients with antibodies for the related type also had antibodies for the homologous type, and the titer of the latter was usually higher than that for the heterologous but related types.

Additional agglutinations were carried out in each serum with from 2 to 8 different strains of Type VIII, with the stock Types I, II and V strains, and with strains of about 15 other types of pneumococci. The results obtained with the various Type VIII strains were remarkably uniform; those with the remaining types were usually negative, even with undiluted sera. Exceptions are noted in the tables.

Among the pneumonia patients were 14 with clinical and x-ray or anatomical evidence of patchy consolidation, which may be termed " atypical " or bronchopneumonia. The findings in these patients were very similar to those obtained in the patients with typical lobar pneumonia.

Of the 14 patients without pneumonia, two had antibodies for the homologous, and one for the related type only. All three of these patients had acute infections of the upper respiratory tract without clinical or

roentgenological evidence of pulmonary consolidation. The titer of antibodies in each of these patients was low.

For each type of pneumococcus, the relationship between the findings of agglutinins and the findings of protective antibodies was similar to that found among cases of Types I and II (7). They are consistent with the concept that, in general, mouse protection is more sensitive than agglutination as an index to type-specific immunity following infection or immunization.

Mixed infections

It was pointed out elsewhere (4) that pneumococci of other types and other significant organisms are found in patients with Types III and VIII infections, particularly the former, more frequently than in pneumonia due to any other of the pneumococcus types. Some of these cases represent concomitant or consecutive infection, but in most of them one or the other organism has no relation to the disease. Antibody studies may aid in determining the possible etiological relationship.

In the present series, 9 cases of mixed infection were studied. Two of these (Cases 6 and 62) represent consecutive infections. The former developed antibodies for 2 types of pneumococcus, in turn, and the latter succumbed to hemolytic streptococcus sepsis after antibodies against Type VIII had developed. In 2 patients (Cases 9 and 58), the Types III and VIII were the significant invaders and the other pneumococci were probably incidental. In the remaining 5 patients (Cases 14, 21, 24, 31 and 70), the Type III or VIII pneumococci or both were probably incidental, as judged by antibody formation. In Case 14, the Type V pneumococcus, against which antibodies developed, could not be isolated from the patient.

Results of absorption experiments

A number of sera in which antibodies were demonstrated for the homologous or the related type or for both were absorbed with both Types III and VIII pneumococci. The effects of such absorption on the agglutinin and protective titers are shown in Table IV. The results were similar for the Type III and the Type VIII patients and corresponded to those obtained in immunized rabbits (3). Absorption with organisms of the homologous type removed the antibodies for these organisms and for pneumococci of the related type, whereas the related organisms absorbed only the antibodies for the same type but not for the type with which the patient was infected.

DISCUSSION

Inasmuch as the typing of pneumococci depends largely on the agglutination reaction, the results obtained with different strains in the several horse antisera are significant. It would seem, on the basis of these find-

TABLE IV

Effect of absorption with Types III and VIII pneumococci on the agglutinins and protective antibodies in serum of patients convalescing from Types III and VIII pneumonia

Case number	Patient	Patients' type	Day of serum	Agglutination with Pn. III				Agglutination with Pn. VIII				Protection against Pn. III				Protection against Pn. VIII			
				Unabsorbed	Absorbed with			Unabsorbed	Absorbed with			Unabsorbed	Absorbed with			Unabsorbed	Absorbed with		
					Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II
4	M. I.	III	19	64	8	64	64	0	—	—	—	10^5	0	10^4	10^3	10^4	0	0	10^1
3	A. S.	III	10	64	0	64	64	0	—	—	—	10^6	0	10^4	—	0	—	—	—
16	J. S.	III	12	0	—	—	—	2	0	0	—	0	—	—	—	10^2	10^2	—	—
	J. S.	III	18	4	—	—	—	2–4	0	0	—	0	—	—	—	10^2	10^1	0	—
7	T. C.	III	33	8	0	0	0	0	—	—	—	10^4	0	10^2	10^1	0	—	—	—
13	F. G.	III	7	0	0	0	0	0	—	—	—	10^4	0	10^3	10^2	0	—	—	—
18	M. K.	VIII	15	2	—	—	—	16	0	0	—	0	—	—	—	10^3	0	—	—
52	S. H.	VIII	14	0	0	0	0	8	4	0	—	10^3	0	1	—	10^1	10^3	0	—
	S. H.	VIII	20	4	0	0	—	4	0	0	—	10^3	0	0	—	10^6	10^6	0	—
53	E. S.	VIII	12	4	0	0	—	4	4	0	—	10^4	0	0	—	10^4	10^4	0	—
51	E. S.	VIII	15	0	0	0	—	8	0	0	—	10^4	0	10^2	—	10^4	10^4	0	—
44	J. McL.	VIII	22	0	—	—	—	16	32	0	8	0	—	—	—	10^5	10^4	0	10^3
46	J. W.	VIII	41	0	—	—	—	2	8	0	0	0	—	—	—	10^5	10^4	0	—
48	L. F.	VIII	16	0	—	—	—	16	4	0	—	10^2	0	0	—	10^6	10^6	0	—
71	E. F.	VIII	10	0	—	—	—	16	—	—	—	10^6	—	—	—	10^5	10^5	0	10^5
	F. D.	VIII	10	0	—	—	—	0	—	—	—	0	0	0	—	0	10^5	0	—
50	C. L.	VIII	16	4	—	—	0	32	—	—	—	10^4	0	0	—	10^4	10^6	0	—

ings, that the choice of a suitable Type III agglutinating serum and additional agglutination, in Type VIII antiserum, of strains reacting with it, should serve to differentiate between these 2 types. Titration in progressive dilutions of both sera are seldom necessary. Prolonged incubation should be avoided. Microscopic agglutination in the same dilution of both antisera gives a rapid and clear differentiation. The precipitin reaction is apparently no more reliable than the agglutination test (3). Type VIII strains, however, do not produce large mucoid colonies on the surface of blood agar plates similar to those characteristic of freshly isolated Type III strains (5). As to the serum, the variations in cross-agglutination observed with different species suggest the possibility that some suitable species will be found in which the Type III immunity is strictly type-specific, as it is in the mouse (3).

The differentiation of these two types is important because of the clinical and pathological differences between the diseases associated with each of these, particularly the wide divergence in death rates, especially in bacteremic patients (4). It may also become important from the therapeutic point of view, inasmuch as all therapy in human pneumococcic infections has thus far been shown to depend on type-specificity. Both therapeutic antisera and carbohydrate splitting enzymes (8) of value in such infections have been shown to be type-specific in their action.

Immune bodies resulting from Type III infections were encountered less frequently and were of lower grade than homologous antibodies resulting from Types I, II or VIII infections. Low grade or absent immune responses are, however, encountered even with Types I and II infections (7, 10). It is not unlikely that instances of transient appearance of antibodies were missed owing to the small number of sera studied. It is also possible that, owing to the frequent finding of Type III pneumococci in normal throats, some of the patients in whom antibodies for this type were not demonstrated were only carriers and the disease was caused by another organism. Such cases were detected by testing the sera with many different types. No satisfactory explanation was found, however, for the failure of an occasional patient to develop antibodies against organisms recovered from the blood.

The present series offered some opportunity to compare the immunity resulting from lobar pneumonia and that following bronchopneumonia due to the same organism. Such opportunities with Types I and II pneumococci must, of necessity, be quite rare owing to the close association of the latter types with lobar pneumonia and the high fatality in the occasional cases of bronchopneumonia due to these types (11). The antibody response with the different kinds of pulmonary lesion due to the same type were very similar. In the patients with simple respiratory infections without pneumonia, antibodies were usually absent or of low titer.

The results of the absorption tests were similar to those obtaining with major and minor antibodies for other related organisms, notably the typhoid-paratyphoid group. In the present cases, they confirm the etiological relationship to pneumonia of Types III and VIII pneumococci obtained from sputum, especially in recovered patients, in whom the same organism usually cannot be obtained from the blood or lungs (10).

SUMMARY AND CONCLUSIONS

Freshly isolated Types III and VIII pneumococci frequently show significant degrees of cross-agglutination in some horse antisera of the related type. The desirability of further aglutinating in Type VIII antiserum strains of pneumococci which react with Type III antisera was emphasized.

The sera of patients with lobar or bronchopneumonia associated with Type III or Type VIII pneumococci have homologous type-specific antibodies similar to those observed following Types I and II pneumococcus pneumonia. In the Type III patients, antibodies were less frequent and of lower titer. Antibodies for the heterologous but related type were found frequently among both the Type III and the Type VIII patients.

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ANTIBODY RESPONSE TO INFECTIONS WITH TYPE II AND THE RELATED TYPE V PNEUMOCOCCUS [1]

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In the preceding communication were reported the results of immunological studies in a group of patients with infections associated with Types III and VIII pneumococci (1). In this paper will be presented similar studies in a group of patients with infections associated with the Types II and V pneumococci. The latter are the most frequent and most important of the pneumococci immunologically related to Type II (2) and correspond to the Group IIa of Avery (3). The materials and the methods were identical with those used in the preceding study.

Agglutination of strains of Types II and V pneumococci in antipneumococcus horse sera of these types

Tests for cross-agglutination were carried out with 10 Type II and 12 Type V strains. One Type V and 3 Type II antisera from different laboratories were available. The Type V serum agglutinated one-half of the homologous strains in dilutions up to 1:80 and the rest up to 1:160 or 1:320. This serum failed to agglutinate 4 Type II strains and agglutinated 6 others in dilutions of 1:10 or 1:20. The Type II sera varied slightly in the titer to which they agglutinated homologous strains; one agglutinated up to 1:40, the second to 1:80 and the third to 1:160. The first failed to agglutinate 8 Type V strains and agglutinated 4 strains in 1:2 or 1:4 dilutions only, the second agglutinated 8 strains in 1:4 dilutions, and 4 others in 1:10 dilution and the third did not agglutinate 6 Type V strains and agglutinated the rest in 1:4 dilutions only.

Microscopic agglutination tests with each strain in 1:10 dilutions of each antisera always showed a clear differentiation. The agglutination with the homologous type antisera was marked. In the related type antiserum agglutination was either absent, or the clumps were small, without much serum surrounding them, and many free unagglutinated organisms were seen.

Thus, cross agglutinations were observed with each of the antisera used, but these were chiefly in the macroscopic tests. There was no defi-

TABLE I

*Antibody response to infection with pneumococcus Type II ***

Case number	Patient	Age	Blood culture		Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Result	Day	Mode	Day		Pn. II	Pn. V	Pn. II	Pn. V	
		years										
72	J. B.	30	Pn. II	2	Crisis	5	3	0	0	0	0	Subcutaneous abscess, left arm, 18th day
			Negative	4			20	32	0	10^7	0	
24	J. O'B.	36	Negative	5	Lysis	10	8	0	0	10^2	0	Pn. III and Pn. VIII (no Pn. II) in sputum on 3rd day.
			Negative	7			10	4	0	—		Pn. II (no Pn. III or VIII) in sputum on 6th day
			Negative	8			12	8	0	10^4	0	
							23	0	0	10^3	0	
73	J. J.	38	Negative	5	Crisis	9	6	0	0	0	0	In the last serum agglutinins (1 : 8) present for both Pn. VIa and Pn. VIb
			Negative	6			9	0	0	0	0	
			Negative	9			12	8	0	10^6	0	
							23	16	0	10^6	0	
74	M. O'D.	42	Pn. II	6	Lysis	7	11	4	0	10^6	10^4	No previous history of pneumonia
							15	16	0	10^6	10^5	
							21	16	0	10^6	10^5	
75	C. S.	47	Negative	5	Crisis	6	9	2	0	10^6	0	
							13	4	0	10^6	0	
76	J. C.	17	Negative	3	Crisis	5	13	8	0	10^5	0	

TABLE I—(continued)

77	C. R.	25	——	—	Lysis	?	18	0	0	0	Prolonged fever, pyelitis, sterile pleural effusion. Pn. III agglutinins (1 : 4), no Pn. III protection
78	W. C.	59	Pn. II Negative	3 9	Lysis	8	5	0	0	0	Later treated with specific serum
79	G. M.	38	Pn. II	2	Died	8	2	0	0	0	Later treated with specific serum
80	T. McD.	37	Pn. II Pn. II	7 9	Died	9	9	0	0	0	
81	D. D.	37	Negative	8	Lysis	8	11	0	0	0	No pulmonary consolidation. Diagnosis: Acute bronchitis

Explanation of Tables I–IV

* The following abbreviations and notations apply to this and subsequent tables of this paper:

Pn. II, Pn. V, etc. = Pneumococcus Type II, pneumococcus Type V, etc.

"Day," = The number represents the number of days from the onset of the disease.

"Agglutinins" = The numbers represent the highest dilution of serum in which floccular agglutination was observed.

"Mouse protection" = The numbers represent the highest number of lethal doses against which mice were protected.   + =
End point not determined.

— = Not determined or test not done.

Except when noted under "Remarks" all patients had lobar pneumonia clinically and by x-ray.   Type II pneumococci were obtained from the sputum on one or more occasions in each case shown in Table I, and Type V in each case shown in Table II.

TABLE II (continued)

Case number	Patient	Age	Blood culture Result	Blood culture Day	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. II	Agglutinins Pn. V	Mouse protection Pn. II	Mouse protection Pn. V	Remarks
89	W. G.	years 27	Pn. V. Negative	5 10	Crisis	8	6 11 18 23	0 0 0 0	2 128 64 64	0 0 0 —	10^3 10^{7+} 10^6 —	
90	L. N.	34	—	—	Pseudo-crisis. Lysis	5 11 21	4 11 31 51	0 0 0 0	0 2–4 2–4 2	0 0 0 0	0 10^4 0 10^5	Secondary rise in temperature 15th to 20th day with extension. Pn. XI recovered from sputum first, Pn. V later. No agglutinins for Pn. XI
91	R. F. S.	61	Pn. V.	7	Crisis	8	8 18	0 0	0 32–64	0 0	0 10^6	
92	V. LaP.	36	—	—	Crisis	11	9 19 22 25	0 2 2 2	4–8 16–32 32–64 32–64	0 10 0 —	10^5 10^6 10^7 —	
93	G. P.	61	Negative Negative	9 12	Lysis	9	11 14 21 28	0 0 0 0	8–16 64 32 16–32	0 0 0 —	10^{6+} 10^{6+} 10^4 10^5	
94	J. L.	40	Negative	8	Crisis	8	8 15 19 24 30 37	0 0 0 0 0 0	8–16 512 128 32–128 64 64	— 0 0 — — 0	— 10^{7+} 10^{7+} — 10^{6+} 10^7	
95	J. McL.	32	—	—	Crisis	8	9 12 17 28	0 0 0 0	64 64 64 16–32	0 — — 0	10^7 — — 10^5	Cirrhosis of liver with jaundice

TABLE II (continued)

Case number	Patient	Age (years)	Blood culture Result	Blood culture Day	Termination Mode	Termination Day	Day of serum	Agglutinins Pn. II	Agglutinins Pn. V	Mouse protection Pn. II	Mouse protection Pn. V	Remarks
96	E. Z.	32	—	—	Lysis	9	14	0	512	0	10^7	
97	A. M.	26	Negative	11	Crisis	9	15	0	128	0	10^6	
98	P. Cl.	33	Negative	8	Crisis	11	14	0	8-16	—	—	
			Negative	9			23	0	128	10^{7+}		
21	P. Ci.	36	Negative	6	Pseudo-crisis	11	12	16-32		10^2		Recurrence of fever 15th to 20th day. Pn. III in sputum 10th day; only Pn. V in sputum 12th and 15th day. No agglutinins or protection for Pn. III or VIII
			Negative	13	Lysis	20	17	8-16		10^1		
						24		2-4		10^2		
						31		2-4		10^2		
99	W. M.	32	Negative	7	Crisis	8	0	0	0	10	0	
						15	0	0	0	10	0	
100	A. F.	36	—		Lysis	7	0	0	0	10^3	0	Common cold, no pneumonia. History of pneumonia (unknown organism) 5 months previously. Irregular continued fever until discharged (against advice)
101	J. H.	21	Negative	4	Lysis	4	0	0	—	—	—	
			Negative	12		8	0	0	—	—	—	
			Negative	15		14	—	—	—	0	—	
			Negative	17								
102	J. R.	46	Pn. V.	9	Died	12	0	0	0	0	0	
			Pn. V.	12		12						
103	C. A.	34	Pn. V.	4	Died	8	0	0	0	0	0	
			Pn. V.	6								
104	P. C.	36	Pn. V.	2	Died	4	0	0	10	0	10	
						3						
105	G. A. K.	40	Negative	2	Died	5	0	0	0	0	0	
			Pn. V.	5		5						

coccus type, to exercise care to exclude Type V cases from among those selected for treatment with Type II antisera. This may be done by further agglutinating in Type V antiserum all strains reactive with Type II.

It is interesting to compare the immune response to infections with Types III and VIII pneumococci (1) with those here observed in Types II and V cases. Homologous type-specific antibodies were more constant and of higher grade with the latter types. With the former, cross-immunity was frequent and of high grade, often associated with higher titers of antibody for the related than for the homologous type and, at times, present in the absence of antibodies for the infecting type. With the latter, cross-immunity was infrequent, of low grade so that it could not be demonstrated by the agglutination reaction, and was always associated with the finding of a high titer of antibodies for the homologous type.

The cases mentioned under the heading of "mixed infections" also present an interesting contrast with the corresponding cases in the Type III group (1). The immunological reactions in the latter suggested that the finding of Type III was incidental in most instances and the other organisms were usually etiologically related to the disease. In the present cases the data suggest an opposite conclusion, namely, that in most instances the other organism was incidental and the Type II or V was the important invader. These findings are not difficult to appreciate in view of the frequency with which Type III organisms are found in the normal respiratory passages, and the rarity with which Types II and V organisms occur under similar conditions (5).

SUMMARY AND CONCLUSIONS

Cross-agglutinations of Types II and V pneumococci in antipneumococcus horse antisera were observed frequently and, in some instances, in dilutions high enough to cause confusion in routine type determinations. The desirability of agglutinating strains of either of these types in both antisera was indicated, especially in relation to the use of specific antisera in treatment.

Homologous type-specific antibodies were found fairly constantly in patients with Type II or V pneumococcus lobar pneumonia who recovered. Fatal patients with either type failed to develop antibodies. Cross-immunity was observed but it was infrequent and of low grade. Where it occurred, absorption experiments indicated that only the organism recovered from the sputum was antigenically active.

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ANTIBODY RESPONSE TO INFECTIONS WITH THE NEWLY CLASSIFIED TYPES OF PNEUMOCOCCI (COOPER) [1]

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The separation of the pneumococci previously included in Group IV into serologically specific types (1) has made possible the accurate identification of nearly all pneumococci. In this paper will be presented the result of studies on the specific antibody response of human subjects to infections associated with these newly classified types of pneumococci. Similar studies in patients with 2 important pairs of immunologically related types of pneumococci, namely, Types III and VIII and Types II and V, have been reported in the preceding communications (2, 3) and only a summary of these results are included here for comparison.

The sera of 190 patients with infections associated with pneumococci other than Type I were studied. The number of patients and sera in each of 3 kinds of cases studied are listed in Table I according to the type of pneumococcus obtained. The " non-pneumonias " include patients with acute and chronic respiratory infections, but without pulmonary consolidation, and those with purulent focal infections. The methods employed were the same as those used in the previous studies (2).

Agglutinins for pneumococci of the homologous type

Agglutinins for the homologous type of pneumococcus were demonstrated in the sera of two-thirds of the patients with lobar or bronchopneumonia associated with Types II and III and with 9 of the newly classified types (see Table II). The data for the individual patients in whose sera such antibodies were demonstrated are shown in Table III and in the corresponding tables in the previous papers (2, 3). Also, 6 of the 22 fatal cases showed agglutinins for the homologous type in the serum before death.

The titer of the agglutinins for the newly-classified types was comparable with that found in similar patients with Types I, II and III pneumococcus pneumonia (4). Sera which agglutinated one strain of a given type also agglutinated all other strains of the same type to approximately the

TABLE I

Material examined

Patients' type	Number of strains	Total number of patients	Lobar pneumonias				Bronchopneumonias				Non-pneumonias*	
			Recovered		Died		Recovered		Died			
			Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera
I	1	4†	3	8	—	—	—	—	—	—	1	1
II	1	11	8	19	2	2	0	—	0	—	1	1
V	4	25	19	68	4	4	1	2	0	—	1	1
III	1	40	19	44	3	5	5	14	3	3	10	17
VIII	8	33	18	65	4	11	4	12	2	3	6	8
IV	3	8	6	16	0	—	0	—	0	—	2	2
VII	4	14	10	30	2	4	2	5	0	—	0	—
IX	2	4	0	—	1	1	0	—	1	2	2	3
XII	2	4	4	11	0	—	0	—	0	—	0	—
XIV	4	8	2	6	1	1	2	3	0	—	3	5
XVII	2	3	2	4	1	1	0	—	0	—	0	—
XVIII	1	4	0	—	0	—	2	6	0	—	2	2
XIX	2	7	3	9	0	—	2	7	0	—	2	2
VI	5	8	2	5	2	3	0	—	0	—	4	9
X	2	4	0	—	1	4	0	—	0	—	3	4
XI	2	2	2	5	0	—	0	—	0	—	0	—
XIII	1	5	1	3	1	1	0	—	0	—	3	3
XV	1	1	1	3	0	—	0	—	0	—	0	—
XX	2	9	5	9	0	—	1	3	0	—	3	3
XXII	1	2	1	2	0	—	1	4	0	—	0	—
XXIX	1	1	0	—	0	—	0	—	0	—	1	1
XXXI	1	1	0	—	0	—	0	—	0	—	1	1
All	51	194‡	106	307	22	37	20	56	6	8	45	63

* Includes acute and chronic respiratory infections without pneumonia, the pneumococcus being obtained from the sputum, and focal pneumococcic infections, where the pneumococcus was obtained from the lesion.

† Each of these had other types of pneumococci in addition to the Type I.

‡ Four patients are listed under 2 separate types.

same titer. In a number of instances, different methods were used to prepare the antigens and carry out the tests, but the results were practically the same in each instance.

In 2 cases, a recovered Type XII and a fatal Type XIV, the same type of pneumococcus was obtained from sputum and blood culture, but agglutinins for the homologous type could not be demonstrated in several sera. No instances were found of homologous type-specific agglutinins for the 9 types shown in the lower portion of Table I, although there were included 14 recovered pneumonia patients from whom these types were obtained.

TABLE II

Results of agglutination tests with homologous type pneumococcus antigens in the sera of pneumonia patients †

Patients' type*	Lobar pneumonia				Bronchopneumonia			
	Recovered		Died		Recovered		Died	
	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive
II	8	6	2	0	0	—	0	—
III	19	11	3	0	5	2	3	2
V	19	17	4	0	1	1	0	—
VIII	18	11	4	0	4	3	2	1
IV	6	5	0	—	0	—	0	—
VII	10	8	2	2	2	2	0	—
XII	4	1	0	—	0	—	0	—
XIV	2	1	1	0	2	1	0	—
XVII	2	0	1	1	0	—	0	—
XVIII	0	—	0	—	2	1	0	—
XIX	3	2	0	—	2	2	0	—
All	91	62	17	3	18	12	5	3

* Only types against which agglutinins were demonstrated in the sera of 1 or more pneumonic patients are given in this table.

† The following abbreviations and notations apply to this and subsequent tables of this paper:

Pn. IV, Pn. VII, etc. = *Pneumococcus Type IV, Pneumococcus Type VII*, etc.

"Day" = The numbers represent the number of days after the onset of the disease.

"Agglutinins" = The numbers represent the highest dilution of serum in which floccular agglutination was observed. More than one number in these columns are recorded when different titers were obtained with different antigens or on repeated tests.

"Mouse protection" = The figures represent the highest number of lethal doses against which mice were protected.

+ = end point not made out.

x = irregular survivals.

— = indeterminate or test not done.

Only 2 patients without pneumonia had homologous type agglutinins. One of these had an acute upper respiratory infection and Type III and the other was a cardiac patient with chronic bronchitis and Type IX pneumococci in the sputum. Others had protective antibodies without agglutinins (2, 3).

Agglutinins for pneumococci of heterologous types

The serum of every patient was tested for agglutinins with Types I, II and III and usually with strains of about 15 heterologous types of

TABLE III

Immune response to infections with the newly classified types of pneumococci
(Only cases with antibodies are listed; Types V and VIII excluded)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
106	A. F.	years 34	IV	Crisis	8	7	0	0	Blood culture negative 6th day. Pn. IV 8th day
						12	8–16	10^3	
						19	4–16	—	
						26	4–16	10^4x	
107	D. P.	58	IV	Lysis	7	11	8	10^4	
						18	4–8	10^4	
108	J. A.	28	IV	Lysis	7	4	8	10^3	
						16	4	10^3	
						25	4	10^3	
109	M. S.	48	IV	Lysis	8	8	8	—	
110	J. P.	25	IV	Crisis	9	7	0	0	
						11	0	0	
						13	2	10^2	
111	C. C.	65	VII	Lysis	7–13	9	0	—	Bronchopneumonia
						11	0	10^3	
						15	16	10^5	
						24	8	10^7	
112	A. G.	18	VII	Lysis	8	9	16	—	
						11	64	—	
						18	64	10^6	
113	A. F.	19	VII	Crisis	8	34	512	10^7	Pn. I pneumonia 3 years previously
114	J. B.	19	VII	Lysis	11	9	0	10^4	
						19	16–64	10^7	
115	J. Z.	37	VII	Crisis	12	14	64	10^5	
116	S. J.	20	VII	Crisis	6	5	0	10	
						10	8–16	10^5	
						15	4	10^3	
						24	0	10^4	
117	A. McD.	43	VII	Lysis	7	4	0	0	
						10	512	10^6	
						15	256	10^7	
						29	32–64	10^6	
						36	32	—	
						43	32	—	
						49	32	10^6	

TABLE III (*continued*)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
		years							
118	M. C.	14	VII	Crisis	9	8	8	—	
						11	8	10^5	
						16	32	—	
						22	16–32	10^{5+}	
119	H. L.	37	VII	Crisis	7	10	64	10^{5+}	Bronchopneumonia
6	D. Van F.	39	VII	Lysis.	23?	25	0	—	(See Table of Type III cases in (2))
				Recurrence	26–34	31	0	0	
						40	4	10^5	
120	P. W.	37	VII	Lysis?	18–28?	22	0	10	Prolonged low-grade fever
						23	0	—	
						25	0	—	
						43	0	10^4x	
						64	0	10^2	
121	H. P.	54	VII	Died	10	9	8–16	10^4	Pseudocrisis 7th day
122	A. McN.	44	VII	Died	32	4	0	10	Pn. VII in blood culture repeatedly to 28th day
						11	2–4	10^6	
						15	16	10^5	
						25	64	10^7	
123	J. O.	44	XII	Crisis	8	5	0	—	
						10	4	—	
						13	8	—	
124	L. A.	62	XIV	Lysis	14	10	0	—	Bronchopneumonia
						21	4–8	—	
125	L. C.	50	XIV	Crisis	8	11	64	—	
						15	256	—	
126	J. L.	42	XVII	Died	13	10	64	—	
127	B. McL.	18	XVIII	Lysis?	5–10	7	4	—	Paratyphoid B infection with positive blood culture on 6th and 7th days
						9	4	—	
						24	8	10^3	
						45	2	—	
						60	0	10	
128	J. F.	53	XVIII	Crisis	16	24	0	10	Bronchopneumonia
129	M. B.	39	XIX	Lysis	7	7	8	—	Bronchopneumonia
						15	0	—	
						23	2	—	
						30	0	—	

TABLE III (*continued*)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
130	E. McD.	*years* 26	XIX	Crisis	4	3	4	—	Bronchopneumonia
						6	8	—	
						10	8	—	
131	J. K.	39	XIX	Crisis	8	10	16	—	
						16	8	—	
132	G. F.	38	XIX	Crisis	12	5	24	—	
						8	4	—	
						20	0	—	
						35	0	—	
133	J. M.	54	IX	—	—	—	8	—	Chronic bronchitis; died 3 months later

pneumococci. There were 18 patients in whom agglutinins were found for types other than those found in the sputum (Table IV). In one-half of these cases, the heterologous agglutinins may be considered to be cross-agglutinations in antisera of types which, from other evidence, appear to be immunologically related. These included 8 instances of cross-agglutination between Types III and VIII and one between Types II and VI.

Results of absorption tests

A number of sera showing agglutinins for the homologous types were absorbed with strains of the same type and with heterologous types of pneumococci. In each instance, the agglutinins were successfully absorbed with pneumococci of the homologous type but were not materially affected by those of heterologous types. These results are shown in Table V and in the corresponding tables in the previous communications (2, 3).

Results of mouse protection tests

Attempts to enhance sufficiently the virulence of a number of strains of the new types of pneumococci for use in protection tests proved unsuccessful, except with Types V and VIII. Virulent strains of Types IV, VII and XVIII pneumococci, however, were obtained through the kindness of Miss Georgia Cooper. Protection tests were carried out on the sera of most of the patients with pneumonia from whom these types were

obtained. The results of similar tests with Types III and VIII and Types II and V have already been reported (2, 3).

The results of the protection tests paralleled, in general, the findings of agglutinins (Table III). Occasional sera showed mouse-protection in the absence of agglutinins for the same type. The titer of antibodies was similar to that observed with the common types.

Mixed infections

In Table VI are listed 12 patients with pneumonia from whom 2 or more organisms, chiefly pneumococci, were isolated. The presence or

TABLE IV

Cases with agglutinins for heterologous type-specific pneumococci

Case number	Patient	Types from sputum	Agglutinins with homologous type	Heterologous type	Heterologous titer	Remarks*
134	C. McD.	XV	0	I	4	Protection against 10^4 L.D., Pn I
135	G. B.	XVII	0	I	16	Pn. XVII in first 2 sputa, B. Friedländer and no pneumococci in a later one
136	H. G.	XX	0	I	4	First sputum showed Hem. Strep. and no pneumococci. Protection for 10^1 L.D., Pn. I
137	A. C.	XXII	0	I	16	Pn. XXII in 2 sputa. No pneumococci in a third
27	D. McC.	III	0	V	4	Fatal case. No protection against Pn. II, III or V. Agglutinins (1 : 2 only) for Pn. II and VIII. Protection 10^2 L.D., Pn. VIII. Blood culture Pn. III
77	C. R.	II	0	III	4	Protected only 1 L.D., Pn. III. No protection Pn. II or V
50	C. L.	VIII	32	III	4	Protection 10^4 L.D., Pn. III and 10^4 L.D., Pn. VIII
43	R. S.	VIII	4	III	4	Protection 10^1 L.D., Pn. VIII; 10^2 L.D., Pn. III
53	E. S.	VIII	8	III	4	Protection 10^5 L.D., Ph. VIII; 10^4 L.D., Pn. III
54	F. H.	VIII	0	III	16	Protection 10^5 L.D., Pn. VIII; 10^5 L.D., Pn. III

TABLE IV (*continued*)

Case number	Patient	Types from sputum	Agglutinins with homologous type	Heterologous type	Heterologous titer	Remarks*
138	L. K.	XII	0	III	8	Protection 10^2 L.D., Pn. III. Also agglutinins (1 : 2) and protection (10 L.D.) Pn. VIII
14	T. A.	III	0	V	8	Protection 10^5 L.D., Pn. V
16	J. S.	III	0	VIII	4	No pneumococci in first sputum. Pn. III in second. Protection 10^5 L.D., Pn. VIII. None for Pn. III
6	D. Van F.	III VII	8 8	VIII	8	First sputum Pn. III. Pn. VII during relapse. Protection 10^3 L.D., Pn. III; 10^6 L.D. for Pn. VII; 10 L.D. for Pn. VIII
18	M. K.	III	2	VIII	16	Protection 10^3 L.D., Pn. VIII; none for Pn. III
3	A. S.	III	64	VII	4	Pn. VII agglutinins in 4th month
73	J. J.	II	16	VI	8	
9	W. L.	III X XVII	4 0 0	VIII	8	Pn. III first, Pn. X and XVII late in convalescence. Protection 10th day 10^4 L.D., Pn. III; O, Pn. VIII; 2 months later O, Pn. III; 10^4 L.D. Pn. VIII

* The titer is given as the greatest dilution in which floccular agglutination was observed.

L.D. = Lethal doses; Hem. Strep. = Hemolytic streptococcus.

absence of serum antibodies for each of these organisms is noted. Antibodies, if present, were usually demonstrated for only one of the organisms. The patients without pneumonia, from whose sputum more than one type of pneumococcus was obtained, are not shown, inasmuch as antibodies were not demonstrated in any of their sera.

Agglutinins and mouse protection in normal subjects

The sera of 26 hospital patients without recent infection or previous history of pneumonia and laboratory workers were tested for agglutinins with all of the types of pneumococci encountered in this investigation. Tests for mouse protection were carried out with Types I, II, III, IV, V, VII and VIII pneumococci. Agglutinins were found in only 2 in-

TABLE V

Absorption of type-specific agglutinins with homologous and heterologous type-specific strains of pneumococci *

Case number	Patient	Patient's type	Day of serum	Titer of homologous type agglutinins				
				Unabsorbed	After absorption with			
					Homologous types	Type I	Other types	Titer
107	D. P.	IV	18	16	0	8	XIV	16
106	A. F.	IV	26	8	0	8	XIX	4
108	J. A.	IV	25	4	0	4	XIX	4
118	M. C.	VII	22	32	0	16	IV	16
							XIV	32
111	C. C.	VII	15	16	0	8	XIV	16
114	J. B.	VII	19	64	0†	—	XIV	32
122	A. McN.	VII	24	64	8–0‡	64	XIX	64
117	A. McD.	VII	10	256	0	128	IV	256
115	J. Z.	VII	14	64	4	64	IV	32
113	A. F.	VII	27	512	64–0‡	512	XIX	256
112	A. G.	VII	11	128	0	128	XI	128
116	S. J.	VII	10	16	0†	64	—	—
123	J. O.	XII	13	8	0	8	VII	8
125	L. C.	XIV	11	32	4–0‡	8	VII	8
131	J. K.	XIX	10	16	0	16	VII	16
129	M. B.	XIX	30	4	0	4	IV	4
130	E. McD.	XIX	10	8	0	8	IV	8

* For absorption of Types II, III, V, and VIII agglutinins see (2) and (3).
† Same result with autogenous and one other strain.
‡ Negative result only after second absorption.

stances: one serum agglutinated Type VII and another Type XIX, each to 1:4 dilution only. Protection against 100 or more lethal doses of the various types occurred as follows: against Type I in one serum; Type II in 2; Type III in 3; Type IV in 5; Type V in 3; Type VII in 19 and Type VIII in 3 subjects. In 6 of the subjects the serum protected against as much as 100,000 lethal doses of Type VII pneumococci. There was no correlation between the presence of protection for any one type and that against any other. These results correspond to those previously obtained with some of these types (5).

DISCUSSION

Strains of pneumococci formerly included in Group IV are found in the nose and throat of most normal individuals (6) and may be obtained, with proper methods, from some patients with pneumonia in whom the Type I or II pneumococcus is the probable invader (7). They are usually less virulent for animals than are strains of Type I and II. Their relationship to lobar pneumonia, therefore, has been doubted (7), especially

TABLE VI

Antibodies in cases of pneumonia with mixed infections *

Case number	Patient	Patient's types	Days isolated	Corresponding antibodies	
				Present or absent	Days
24	J. O'B.	III, VIII	4	Absent	8, 10, 12, 23
		II	7	Present	10, 12
21	P. Ci.	III	11	Absent	12, 17, 24, 31
		V	16	Present	12, 17, 24, 31
90	L. N.	XI	3	Absent	4, 11, 31, 51
		V	21	Present	11, 31, 51
6	D. Van F.	III	22	Present	25, 31, 40
		VII	30	Present	40
9	W. L.‡	III	5	Present	3, 12
		X	82	Absent	3, 12, 83, 94
		XVII	86	Absent	3, 12, 83, 94
58	R. J.	VIII	6 (B.C.)†	Absent	7, 9, 15, 27
		XVIII	12	Absent	7, 9, 15, 27
109	M. S.	IV	1	Present	8
		B. mucosus capsulatus, Type A	1	Absent	8
127	B. McL.§	XVIII	9	Present	7, 9
		B. paratyphosus B	6, 7 (B.C.)	Present	7, 9, 24, 45, 60
139	H. D.	XIX	6, 8	Absent	7, 10, 18
		XX	6	Absent	7, 10, 18
		I	8	Present	7, 10, 18
140	G. O.‖	X	5, 11	Absent	5, 9, 10, 12
		I	8, 13 (B.C.)	Absent	5, 9, 10, 12
141	C. R.	XX	6	Absent	16, 24, 32
		I	15	Absent	16, 24, 32
62	E. H.¶	VIII	4	Present	9
		Strep. hem.; Staph. aur.	10 (P.M.)*	——	No tests

* Patients with 2 types of pneumococci or with other significant organisms. See also Table IV.

† (B.C.) = organism from blood culture only; all others are from sputum.

‡ Only *Streptococcus viridans* from sputum on 6th day. No significant organisms from sputum on 19th and 33d days.

§ Only *Streptococcus viridans* from sputum on 6th day.

‖ No pneumococci from sputum on 5th day. Died 13th day.

¶ Agglutinins and protection for Pn. VIII absent 5th day. Blood cultures negative 5th and 9th day. Autopsy cultures: Heart's blood: *Strep. hem.;* lungs: *Strep. hem.* and *Staph. aureus.*

* (P.M.) = Cultured at postmortem.

in the favorable cases, since bacteremia in such cases is rare and lung punctures usually yield negative results (8).

In the present study, the sera of patients with infections associated with the newly classified types of pneumococci were tested for antibodies reactive with pneumococci of the homologous type and with most of the other types commonly encountered. The objects of these tests were (1)

to verify the antigenic, and, therefore, also the probable etiologic relationship of the newly classified types of pneumococci to the diseases with which they are associated, (2) to determine the specificity of this relationship, (3) to determine whether, in cases where the serum fails to react with the homologous type, another pneumococcus, not recovered from the patient by the ordinary methods, was responsible for the infection, and (4) to shed some light on the significance of the finding of more than one type in the same patient.

The results of these tests and of the absorption experiments indicate that, in most instances, these newly classified types of pneumococci are comparable to Types I and II in their antigenic relationship to the diseases in which they are found. The rarity with which Types I and II antibodies were demonstrated in these cases is worthy of note, since every serum was tested with these types. These types cannot be considered the exclusive causes of lobar pneumonia.

Protective antibodies, frequently of high titer, against some types of pneumococci, were demonstrated in many normal individuals. These findings, however, do not vitiate the results obtained in the pneumonia patients, since only the latter showed agglutinins and since the tests early in the disease were usually negative.

SUMMARY AND CONCLUSIONS

Two-thirds of the recovered pneumonia patients with 11 different types of pneumococci, including 9 of the newly classified types (Cooper), were found to have antibodies for the homologous type of pneumococcus. The results were similar in the patients with bronchopneumonia and those with lobar pneumonia. A number of fatal patients also had such antibodies late in the disease. Occasional patients with acute respiratory infections, without demonstrable pneumonia, also showed antibodies for the type of pneumococcus recovered from their sputa.

The specificity of the immune response was demonstrated by the failure, in such instances, to find antibodies for the other common types and by the specific absorption of the antibodies with pneumococci of the homologous and not the heterologous type.

A few cases were found to have antibodies against types other than the one isolated from the patient's sputum. Most of these represented cross-agglutinations with related types, particularly Types III and VIII.

In the patients in whom more than one type of pneumococcus was isolated, immunity was usually present against only one of these.

These findings tend to confirm the biological identity of the newly classified types of pneumococci. They lend further support to the etiological relationship of these types to the acute pulmonary infections with which they are associated.

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THE RELATION BETWEEN PHYSICAL CONSTITUTION AND THE INCIDENCE OF DISEASE

THE DISEASE GROUPS INCLUDE PEPTIC ULCER, CHOLECYSTITIS AND DIABETES MELLITUS

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HISTORICAL SUMMARY

The problem associating constitution with the occurrence of disease in individuals is a very old one. It is probably as old as the study of disease itself, and its importance must have become impressed upon the minds of men investigating disease from the earliest times. Scrutiny of the Hippocratic writings (460 B.C.–375 B.C.), however, does not reveal any definite allusion to the relation between constitution and disease, although there are vague hints that these two elements are related in Hippocrates' mind. Any direct reference to this subject is likewise lacking in Galen's work (A.D. 130–200). Avicenna (circ. A.D. 980–1037) in his Canon does make numerous allusions to the importance of a recognition of the "temperament" in the study of character and in defining certain functions in an individual, but he does not correlate form with disease. The following sentences are worth quoting inasmuch as they indicate the status which the study of constitution had reached about A.D. 1000, a rather uncertain period in medical scientific history (1). "The external configuration of the body, including the physiognomy, is a reflection of the functional capacity of the internal organs and general make-up of the individual. The character, talents, physical form, shape of individual features, general development and indeed every detail of the physique, length of limbs, of fingers, cutaneous markings, contour of the eyes and ears etc., are all part and parcel with the functional conformation of the viscera and the mental characters." An interesting opinion on the relation between function and form is provided by Walkington in the "Optick Glasse of Humors" (1663). This author takes great pains to point out all the information that can be gained by physiognomical study. In this respect Walkington may be regarded as a precursor to that psychiatric school which links up certain constitutional types with psychiatric disease. The great clinical teachers Sydenham (1624–1689) and Boerhaave (1668–1738) did not add any new contributions to the subject.

There was renewed awakening of interest in the problem at the beginning of the nineteenth century, with an enthusiasm which was sustained until Pasteur's bacteriologic work eclipsed all else for the time in medicine. A rich literature grew up on the continent, while the group of celebrated English clinicians of the nineteenth century left some lasting contributions. Jonathan Hutchinson was perhaps the keenest student of constitution in this group. He possessed a wide background of clinical training, and he could therefore draw upon this experi-

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ence in arriving at his conclusions.   Although Hutchinson is often cited as an authority who pointed towards the close relationship between constitution and certain diseases, actually study of his work discloses that he was skeptical as to the existence of such a relationship.   In "The Pedigree of Disease" (6) Hutchinson points out in quite a modern fashion the difficulties inherent in a study of constitution.   "He (the student of constitution) will discover that he is mistaking for criteria of temperament conditions which are simply indicative of *youth*,[1] or *age*, or *health* or *disease*, or the effects of past anxiety and trouble. . . . So should the student of temperament scrupulously reject all that has been superadded and is in a sense accidental. . . . Indeed it may be questioned whether in a large majority of cases there do really exist in persons as yet in perfect health any peculiarities by which we can predicate or discriminate the
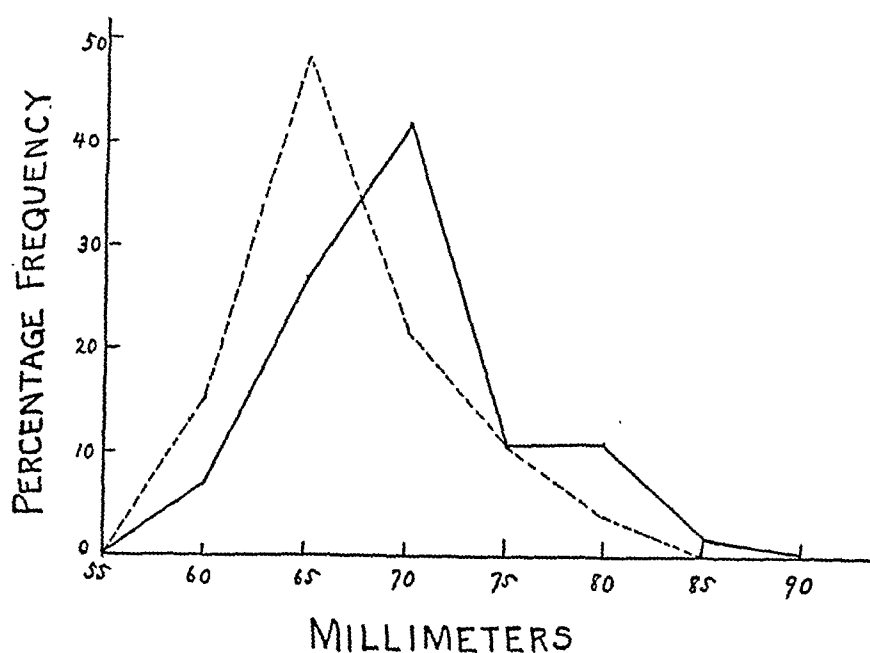

FIG. 1

Nasion prosthion (male).   ——————— Peptic ulcer cases 55; ———————— Diabetic cases 27.   Note (*a*) the peak of the ulcer patients at 70 millimeters and the peak for the diabetics at 65 millimeters, (*b*) the very considerable area of overlapping which may be seen in almost each one of the curves.   In this instance 't' was significant.

'fundamental mode of vital activity (constitution)'."   Furthermore, he shrewdly called attention to the importance of race as a factor which might easily interfere with the correct interpretation of any results obtained in a study of constitution.

For a period of 50 or 60 years following Pasteur's initial endeavours, the study of constitution was neglected, but interest in it was revived once more at the beginning of the present century.   The reason for this was that the available bacteriological knowledge was found inadequate to explain many of the phenomena of disease so that interest shifted from micro-organisms to man himself.   Most prominent in the recent investigation of constitution have been

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[1] Italics our own.

Bauer (12), Kretschmer (13), di Giovanni (15), Stockard (8) and Draper (5). Draper has been one of the most conspicuous workers in this field. Moreover, he deserves much of the credit for applying exact mathematical methods to the investigation of a problem which in the past was approached from the point of view of indefinite clinical impressions. In the present work we have pursued Draper's suggestions insofar as the anthropometric measurements are concerned.

THE PROBLEM AND THE METHODS EMPLOYED

Constitution may be defined as the sum total of native or acquired characteristics by virtue of which the individual is rendered more or less susceptible to disease. Most of the confusion in the study of constitution has arisen because of the failure to divide the subject into its component parts,
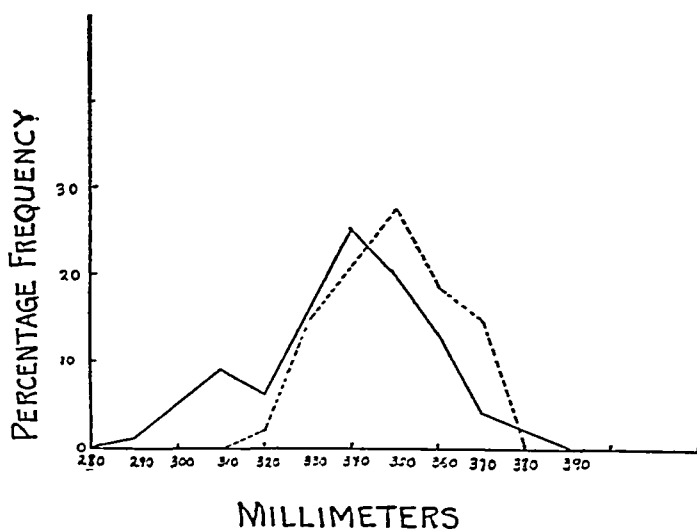

MILLIMETERS

FIG. 2

Neck circumference (male). ———— Peptic ulcer cases 55; — — — — Diabetic cases 27. The peptic ulcer curve is skewed to the left, indicating that the neck circumference in this disease group is smaller than in the diabetic patients.

to reach a sound conclusion regarding each of these parts, and then to correlate all the phases of the subject. If the problem were attacked from the point of view of each constituent phase, one would then be prepared to decide how constitution may affect the incidence of disease in man. Draper has divided human constitution so far as it relates to disease into four parts—the anatomical, physiological, immunological and psychological panels. His early work dealt with the first of these panels, and the present work has been restricted to this field. It is this aspect of constitution which caught the fancy of clinicians through the ages. It is this aspect also which lends itself, in many ways at least, to more or less accurate methods of

study.   It should be emphasized however, that a negative result in the anatomical panel does not invalidate the observations that man and animals show a predisposition or an immunity to certain diseases.   Failure to obtain confirmation of this fact by a careful study of the external configuration of the body indicates only that an old clinical theory is not founded on actual fact.

The problem presented by an investigation of the anatomical panel simply stated appears to be this—will an individual of a given complexion with certain body measurements and proportions be especially predisposed to the development of a specified disease?   The difficulties encountered
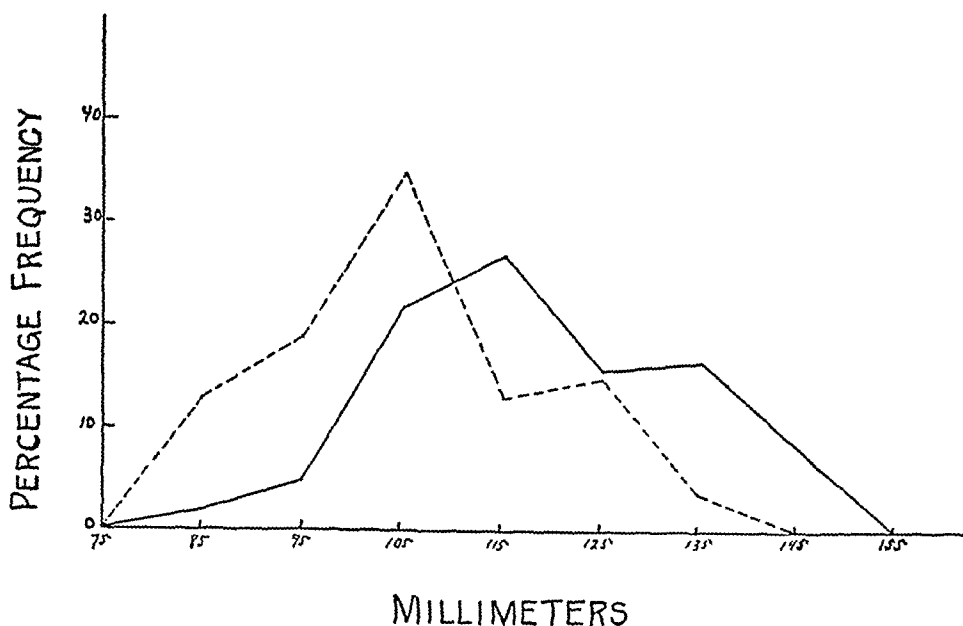
MILLIMETERS

FIG. 3

Neck length (male).   ——————— Peptic ulcer cases 55; ——— ——— Diabetic cases 26.   Note the relatively small area of overlapping in this figure.   Statistical treatment of the data involved showed a well marked tendency for peptic ulcer males to display a longer neck; 't' in this case was 3.8, almost twice the limit of significance.   It is in a case of this kind that the relative frequency curve suggests the actual state of affairs.

in working out this problem are as follows.   Firstly, one must be reasonably sure that certain body proportions are not *caused* by disease.   For this reason we have avoided selecting diseases which are characterized especially by cachexia.   Furthermore, most of the measurements which have been taken are those of the distances between bony points, and disease except in unusual instances would be unlikely to affect these.

Secondly, the racial factor is a very important one in evaluating the importance of the physical constitution.   However, as will be seen, the racial distribution of the cases did not affect our results significantly.   In the present discussion of race, it was not possible to divide patients as be-

longing to the ethnologic groups typified by the Alpine, Mediterranean, Scandinavian and Slav races. The most that could be done was to record and note the country of birth of each patient and employ this in the loose sense of " race." In the three large disease groups there was no predominance of one or other racial type, but each group showed approximately the same mixture of races as did the other. Thus in the male diabetic, peptic ulcer, and cholecystitis groups, the greatest percentage was of Canadian birth (Table I). In the diabetic female group also, those of Ca-

TABLE I

Racial distribution of disease groups

Percentages

Racial types	Dia-betes, male	Peptic ulcer, male	Chole-cystitis, male*	Dia-betes, female	Peptic ulcer, female†	Chole-cystitis, female
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Canadian....................	51	23	44	29	25	14
English......................	8	17	22	8	33	14
Scottish.....................	0	16	0	0	17	5
Irish........................	8	3	0	0	0	0
Russian Jewish................	15	5	22	31	17	24
Polish Jewish.................	0	17	0	6	0	11
Roumanian Jewish.............	8	3	12	11	0	3
Slav........................	0	9	0	2	0	5
French Canadian..............	7	0	0	6	0	16
American....................	0	2	0	3	0	3
Mediterranean................	0	2	0	2	0	5
Miscellaneous................	3	3	0	2	13	0

* Total, 9 cases.

† Total, 12 cases.

nadian origin were second only to those of Russian birth. Again, although in the female peptic ulcer and cholecystitis groups native English and Russians predominated, nevertheless, Canadians constituted a relatively high percentage. The ideal condition, of course, would be to select individuals of the disease groups from a single pure race. Unfortunately, such a selection would be almost impossible in Canada, and it would be very difficult of realization even in Europe.

A third factor which must be borne in mind, and for which allowance should be made, is the discrepancy in results which may appear because of a difference in the number of individuals in the various disease groups (Table II). It will be evident that the number of cases in two of the disease entities was small. There were 9 patients in the male cholecystitis group, and 12 patients in the female peptic ulcer group. However by the statistical treatment of the figures this difficulty was overcome in part at least. In the review of the figures, those errors which were due to " sample

TABLE II

Number of cases employed

Disease group	Male	Female
Diabetes	27	52
Peptic ulcer	55	12
Cholecystitis	9	37

differences," that is discrepancies which occurred because of the employment of groups of small size, usually could be differentiated from actual differences.

Particular care was exercised in the selection of cases, so that only those patients in whom the diagnosis was certain were used in the work. In the case of diabetics, the presence of symptoms with hyperglycemia and glycosuria were the criteria of diagnosis. Practically all the patients in the cholecystitis group showed cholecystitis with cholelithiasis at operation before being included in the series. The large majority of patients in the peptic ulcer group showed corroboration of the diagnosis by operation. In a few, the diagnosis was made by a combination of the clinical picture and the roentgenographic findings.

The actual anthropometric work was carried out by one person (J. F.), so that the important source of error which may arise in work of this kind as a result of the personal equation, was eliminated. As already stated, we followed in broad outline Draper's methods, although the number of measurements was reduced considerably and usually the distance between bony points was preferred. It was not always possible to be certain that the anthropological points selected in this work coincided precisely with Draper's, and this probably accounts for many of the more striking differences in our results. Altogether, 37 measurements were taken on each patient, in addition to notes regarding the individual's complexion, distribution of hair, colour of the iris, and presence or absence of freckles, but only the anthropological measurements will be considered at this time. Table III indicates how the various measurements were obtained.

The lower age limit decided upon for the statistics studied was 21 years, because most of the bones have ceased growing by this time. Table IV indicates the average age of the individual disease groups. It will be noted that the greatest difference between any two groups is 11.5 years. Moreover this difference occurs in the fifth decade of life, so that its significance, so far as anthropometric data is concerned, is very slight.

So far as the application of the statistical method to the work is concerned, certain well-defined rules were followed. The mean (or average) was first obtained for each of the groups. The difference between the means was then obtained, and the standard error of *this difference* determined. Where the difference between the means was equal to or exceeded twice the standard error, the ratio (indicated in this paper by the letter " t ") was regarded as significant. That difference then may be expressed

TABLE III

Anatomic points used to obtain measurements

Facial diameter: Distance between the most prominent points of origin of maxillary ridges.

Nasion prosthion: Distance between bridge of nose and interval between incisors.

Ear width: From the tip of the tragus to the outermost part of the ear.

Ear length: From the tip of the lobe to the uppermost part of the helix.

Ascending ramus length: From the angle of the mandible to the tip of the coranoid process.

Horizontal ramus length: From the angle of the mandible to the symphysis.

Bigonial diameter: The distance between both angles of the mandible.

Inter-pupillary space: The distance between the center of each pupil, with the patient looking straight ahead.

Palpebral breadth: The distance between the free margin of the lids with the patient looking straight ahead.

Palpebral length: The length of the free margin of the lids, with the eyes shut.

Cephalic length: The distance between the occiput and the most prominent part of the glabella as obtained by a cephalometer in the mid-sagittal plane.

Cephalic breadth: The distance between the most prominent parts of the parietal bones as measured by a cephalometer, at right angles to the mid-sagittal plane.

Nasion submenton: The distance between the bridge of the nose, and the under surface of the symphysis of the mandible.

Neck circumference: Circumference at the base of the neck.

Neck length: Distance between episternal notch and the junction between the neck and chin, as measured by a cephalometer.

Thoracic anteroposterior diameter: The antero-posterior diameter at the level of the 4th rib anteriorly.

Thoracic lateral diameter: The lateral diameter at the level of the 4th interspace anteriorly.

Bi-acromial diameter: The distance between the most prominent parts of the acromial processes.

Chest circumference: Circumference of the chest at level of the 4th interspace.

Chest length: Episternal notch to tip of manubrium.

Umbilicus-xiphoid: Distance between umbilicus and tip of xiphoid.

Umbilicus-pubis: Distance between umbilicus and the upper level of the symphysis pubis.

Bi-iliac diameter: Distance between anterior superior spines.

Nail length: Longitudinal diameter of nail bed.

Nail breadth: Transverse diameter of nail bed.

Hand length: Distance between the end of the radial styloid and the tip of the middle finger.

Hand breadth: The widest part of the palm.

Finger circumference: Circumference at the junction of first and second phalanges.

Leg circumference: At the middle of the lower leg.

Acromion to the lateral epicondyle of the humerus: Bony points as stated, giving length of humerus.

Lateral epicondyle of the humerus to the tip of radial styloid: Bony points as stated, giving length of radius.

Acromion to the tip of the middle finger: As stated.

Internal meniscus of the knee to the tip of tibia: As stated, giving length of tibia.

Great trochanter of the femur to the lateral meniscus: As stated, giving length of femur.

Great trochanter of the femur to the ground: Tip of the great trochanter to ground, giving total length of lower limb.

numerically by saying that the chances of an individual with such a body measurement falling into a certain disease group is twenty to one. This

TABLE IV

Age distribution of disease groups

Disease group	Sex	Average age years
Diabetes......................	Male	49.5
Diabetes......................	Female	52.3
Peptic ulcer..................	Male	43.2
Peptic ulcer..................	Female	44.1
Cholecystitis.................	Male	52.7
Cholecystitis.................	Female	40.8

method of working out results is applicable to groups of 30 or over, and it can be applied with less certainty to smaller groups. The equation involved may be represented thus:

$$``\text{t}" = \frac{\text{difference of mean}}{\text{standard error of difference}}.$$

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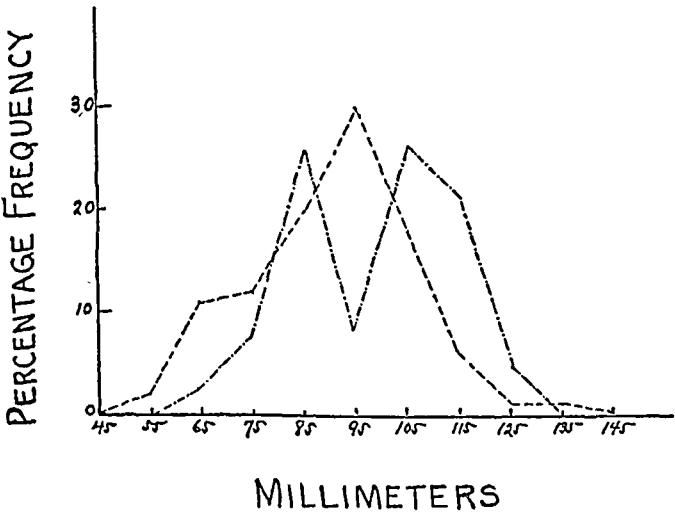
FIG. 4

Neck length (female). — — — — Diabetic cases 52; — · — · — · Cholecystitis cases 37. Note the irregularity of the cholecystitis curve. Such a curve indicates "sample errors," if the number of cases employed is small. It is not usual for two peaks to occur in the same curve in a biometric graph.

It was thought also that if the result obtained in this work were to be subjected to more critical analysis we would be much closer to the actual state of affairs. *Consequently in evaluating the importance of " t " only those differences were considered significant in which " t " was 2 or higher in both the male and the female groups.* Finally, it is important to emphasize the fact that in all this work, one is dealing with whole groups, so that the application of certain conclusions, which may be perfectly valid for *groups,* is hazardous when only one *individual* is concerned.

The graphic representation of statistical results in this as in other problems, although attractive, cannot be accepted as the final test of the statisti-

cal method. Graphic figures are useful for creating a visual image of the distribution of certain numbers over a given range. However, when it becomes necessary to *compare* two or more sets of numbers, and to designate the importance of this comparison, then the graphic method fails. It is for this reason that relatively few graphs are presented here, and these only in the cases where " t " was found to be significant. It was necessary in constructing graphs to choose one of several methods generally employed. *Cumulative frequency curves* have been used by other workers in this field. In the present work *relative frequency curves* were constructed largely because it was considered that they offer a more accurate representation of facts than do the cumulative curves. It is unfortunate that Draper's curves and ours cannot be compared because of the difference in the type of curve employed.

RESULTS

So far as the results are concerned, these have been set down in Table V. This table really constitutes a concise summary of the work presented inasmuch as in it are found the averages for each group, the differences between the averages, and the value for 270 of the measurements including 48 indices. Of the total number " t " equalled or exceeded 2, in 53 instances, or 19 per cent. The difference observed in the nasion prosthion between the peptic ulcer and cholecystitis males in this series is not seen in Draper's figures. The bigonial diameter, the measurement which determines the lower facial width, is greater in both the male and female diabetic groups than in the peptic ulcer cases. This is one of the few examples of a difference occurring in the same direction and of significant degree in both the male and female members of the disease groups.

The neck length of the peptic ulcer male and female groups was definitely greater than in the diabetic patients, while a similar difference was observed in comparing the cholecystitis and the diabetic females.

The thoracic anteroposterior diameter was deeper in the diabetic than in the peptic ulcer groups, both with the males and the females. The same relationship was discovered to exist in the thoracic lateral diameter and in the chest circumference. And so it can be stated that the diabetic population has a deeper, a broader and a more voluminous chest than the peptic ulcer race.

The bi-acromial diameter of the peptic ulcer females was greater than the corresponding diameter of the cholecystitis group. Draper's figures showed an inverse relationship. In this case where the distance measured lay between bony points, the failure of these figures to corroborate those of Draper indicates that the association between a large bi-acromial diameter and peptic ulcer females is not a constant one, and that we are dealing here with a " sample error." On the other hand, the results in this and in Draper's series are comparable when applied to the chest circumference in

TABLE V

Arithmetic means, difference of means, value of t

Part measured	Arithmetic mean, M_1. Peptic ulcers	Arithmetic mean, M_2. Diabetics	Arithmetic mean, M_3. Cholecystitis	Difference between M_1 and M_2, t_{1-2}	Difference between M_1 and M_3, t_{1-3}	Difference between M_2 and M_3, t_{2-3}
Facial diameter, *mm.*						
M	110.5	111.8	112.1	−1.3; 0.8	−1.6; 0.7	−0.3; 0.07
F	101.8	106.2	105.3	−4.4; *2.3*	−3.5; 1.8	0.9; 0.6
Nasion prosthion, *mm.*						
M	70.0	67.0	66.0	3.0; *2.4*	4.0; *2.1*	1.0; 0.5
F	62.8	62.0	63.9	0.8; 0.4	−1.1; 0.6	−1.9; 1.7
Ear width, *mm.*						
M	29.4	29.1	28.1	0.3; 0.5	1.3; 1.4	1.0; 0.8
F	27.1	27.8	27.1	−0.7; 0.7	0.0; 0.0	0.7; 1.1
Ear length, *mm.*						
M	66.6	66.7	68.8	−0.1; 0.1	−2.2; 1.4	−2.1; 1.2
F	59.5	64.5	61.3	−5.0; *3.1*	−1.8; 1.1	3.2; *2.8*
Ascending ramus; length of mandible, *mm.*						
M	55.0	56.8	58.5	−1.8; 1.2	−3.5; 1.6	−1.7; 0.6
F	49.0	51.1	51.0	−2.1; 1.2	−2.0; 1.2	0.0; .04
Horizontal ramus; length of mandible, *mm.*						
M	94.2	94.5	97.6	−0.3; 0.2	−3.4; 1.7	−3.1; 1.3
F	86.6	90.4	86.8	−3.8; *2.4*	−0.2; 0.0	3.6; *2.8*
Bigonial diameter, *mm.*						
M	105.0	108.5	111.5	−3.5; *2.4*	−6.5; *3.3*	−3.0; 1.1
F	98.4	102.8	101.5	−4.4; *2.6*	−3.1; 1.6	1.3; 1.1
Interpupillary space, *mm.*						
M	64.0	63.9	65.0	0.09; 0.08	−0.9; 0.5	−1.0; 0.5
F	98.4	102.8	101.5	4.4; *2.6*	−3.1; 1.6	1.3; 1.1
Palpebral length, *mm.*						
M	27.8	28.2	27.2	−0.4; 0.3	0.6; 0.4	1.0; 0.7
F	27.8	27.7	26.8	0.1; 0.05	1.0; 0.8	0.9; 1.1
Palpebral breadth, *mm.*						
M	10.7	10.0	10.3	0.7; 1.4	0.4; 0.5	−0.3; 0.4
F	9.4	9.8	10.2	−0.4; 0.7	−0.8; 1.8	−0.4; 1.1
Cephalic length, *mm.*						
M	180.2	182.1	179.2	−1.9; 1.0	1.0; 0.3	2.9; 1.0
F	174.0	173.1	172.5	0.9; 0.4	1.5; 0.6	0.6; 0.3
Cephalic breadth, *mm.*						
M	143.6	145.2	145.4	−1.6; 1.0	−1.8; 0.7	−0.2; 0.0
F	139.5	143.4	142.3	−3.9; 1.8	−2.8; 1.6	0.9; 0.6
Nasion submenton, *mm.*						
M	118.0	116.0	116.3	2.0; 1.1	1.7; 0.6	−0.3; 0.0
F	106.0	106.9	105.9	−0.9; 0.4	0.1; 0.0	1.0; 0.7
Neck circumference, *mm.*						
M	338.4	349.0	342.7	−10.6; *2.6*	−4.3; 0.6	6.3; 1.0
F	317.9	329.3	324.3	−11.4; 1.5	−6.4; 0.8	5.0; 0.9
Neck length, *mm.*						
M	119.0	105.9	108.4	13.1; *3.8*	10.6; 1.9	−2.5; 0.4
F	101.4	90.3	98.1	11.1; *2.3*	3.3; 0.6	−7.8; *2.3*
Thoracic anteroposterior diameter, *mm.*						
M	195.4	215.9	203.8	−20.4; 4.5	−8.4; 1.3	12.0; 1.3
F	175.4	200.1	184.0	−24.7; *3.5*	−8.6; 1.4	16.1; 3.5

TABLE V (continued)

Part measured	Arithmetic mean, M₁. Peptic ulcers	Arithmetic mean, M₂. Diabetics	Arithmetic mean, M₃. Cholecystitis	Difference between M_1 and M_2, t_{1-2}	Difference between M_1 and M_3, t_{1-3}	Difference between M_2 and M_3, t_{2-3}
Thoracic lateral diameter, *mm.*						
M	265.8	280.4	273.0	−14.6; 2.8	−7.2; 1.0	7.4; 0.6
F	238.7	255.2	256.9	−16.5; 2.2	−18.2; 3.0	−1.7; 0.3
Bi-acromial diameter, *mm.*						
M	380.4	386.8	374.7	−6.4; 1.2	5.7; 0.6	12.1; 1.2
F	352.0	337.7	339.2	14.3; 2.5	12.8; 2.2	1.5; 0.4
Thoracic circumference, *mm.*						
M	818.0	869.0	868.8	−51.0; 3.6	−50.8; 2.9	0.2; 0.05
F	759.1	827.0	818.1	−67.9; 2.6	−59.0; 2.5	8.9; 0.5
Thoracic length, *mm.*						
M	181.5	177.9	190.0	3.5; 1.0	8.5; 1.5	12.1; 2.0
F	164.5	167.5	164.5	−3.0; 0.5	−0.0; 0.0	3.0; 0.9
Umbilical-xiphoid, *mm.*						
M	142.4	155.1	148.1	−12.7; 2.4	−5.7; 0.6	7.0; 0.7
F	155.0	169.8	158.2	−14.8; 1.5	−3.2; 0.3	11.6; 1.6
Umbilical-public, *mm.*						
M	144.8	144.2	140.6	0.6; 0.1	4.2; 0.6	3.6; 0.5
F	150.0	150.4	149.8	−0.4; 0.7	0.2; 0.0	0.6; 0.1
Bi-iliac diameter, *mm.*						
M	233.2	241.4	234.1	−8.2; 1.7	−0.9; 0.1	7.3; 1.2
F	233.7	240.4	236.3	−6.7; 1.0	2.6; 0.5	4.1; 1.0
Nail length, *mm.*						
M	12.8	12.1	13.0	0.7; 1.3	−0.2; 0.1	−0.9; 1.0
F	10.7	10.7	10.2	0; 0	0.5; 0.8	0.5; 1.3
Nail breadth, *mm.*						
M	16.2	15.0	16.3	1.2; 3.6	−0.1; 0.1	−1.3; 2.8
F	13.7	13.9	13.9	−0.2; 0.4	−0.2; 0.4	0.0; 0.0
Hand length, *mm.*						
M	188.6	185.3	189.3	3.3; 1.9	−0.7; 0.2	−4.0; 1.2
F	172.5	169.7	168.2	2.8; 0.9	4.2; 1.5	1.4; 0.8
Hand breadth, *mm.*						
M	82.2	80.7	81.8	1.5; 1.2	0.4; 0.1	−1.1; 0.5
F	74.1	73.5	71.7	0.6; 0.4	2.4; 1.7	1.8; 2.0
Finger circumference, *mm.*	60.0	60.5	62.0	−0.5; 0.5	−2.0; 1.2	−1.5; 1.0
F	54.8	56.1	54.6	−1.3; 0.7	0.2; 0.1	1.5; 1.4
Leg circumference, *mm.*						
M	289.3	302.2	299.3	−12.9; 2.2	−10.0; 1.0	2.9; 0.3
F	290.8	294.4	301.6	−3.6; 0.3	−10.8; 1.1	−7.2; 1.0
Humerus, *mm.*						
M	301.0	301.6	308.5	−0.6; 0.1	−7.5; 1.0	−6.9; 0.9
F	281.3	279.7	277.2	1.6; 0.2	4.1; 0.7	2.5; 0.6
Radius, *mm.*						
M	261.6	256.6	255.0	5.0; 1.1	6.6; 0.9	1.6; 0.2
F	233.1	232.3	225.1	0.8; 0.1	8.0; 1.4	7.2; 2.0
Arm length, *mm.*						
M	748.2	739.5	753.3	8.7; 1.1	−4.9; 0.4	−13.8; 0.8
F	677.7	672.3	663.3	5.4; 0.4	14.4; 1.2	9.0; 1.1
Femur, *mm.*						
M	406.1	397.1	416.4	9.0; 1.4	−10.3; 1.0	−19.3; 1.8
F	388.1	376.9	370.0	11.2; 1.1	18.1; 1.8	6.9; 1.0

TABLE V *(continued)*

Part measured	Arithmetic mean, M_1. Peptic ulcers	Arithmetic mean, M_2. Diabetics	Arithmetic mean, M_3. Cholecystitis	Difference between M_1 and M_2, t_{1-2}	Difference between M_1 and M_3, t_{1-3}	Difference between M_2 and M_3, t_{2-3}
Tibia, *mm.*						
M	398.5	389.5	405.7	9.0; 1.5	−7.2; 0.7	−16.2; 1.4
F	370.4	360.6	352.1	9.8; 1.3	18.3; *2.1*	8.5; 1.5
Total leg length, *mm.*						
M	877.3	864.1	898.5	13.2; 1.2	−21.2; 1.2	−34.4; *1.7*
F	813.6	800.0	785.1	13.6; 0.8	28.5; 1.7	14.9; 1.4
Foot height, *mm.*						
M	72.4	77.6	76.4	−5.2; 1.1	−4.0; 0.5	1.2; 0.1
F	55.0	62.3	64.6	−7.3; 9.9	−9.6; 1.2	−2.3; 0.4
Height, *inches*						
M	65.7	66.4	68.1	−0.7; 0.9	−2.4; 1.9	−1.7; 1.2
F	61.5	60.9	60.6	0.6; 0.6	0.9; 0.8	0.3; 0.4
Weight, *pounds*						
M	128.4	147.0	146.6	−18.6; *3.9*	−18.2; *2.4*	0.4; 0.3
F	125.6	136.9	138.6	−11.3; 1.0	−13.0; 1.5	−1.7; 0.2
Upper facial index *						
M	63.7	60.3	58.9	3.4; 1.8	4.8; *2.3*	1.4; 0.3
F	61.8	58.5	61.0	3.3; 1.9	0.8; 0.3	−2.5; *2.1*
Lower facial index, †						
M	112.6	107.3	104.1	5.3; *2.3*	8.5; *2.6*	3.2; 0.9
F	107.7	104.1	104.4	3.6; 1.6	3.3; 1.2	−0.3; 0.1
Neck index ‡						
M	35.2	30.6	31.7	4.6; *4.3*	3.5; *2.1*	−1.1; 0.6
F	32.2	27.6	30.5	4.6; *2.6*	1.7; *8.3*	−2.9; *2.3*
Thoracic index 1§						
M	73.8	77.4	75.6	−3.6; *2.4*	−1.8; 0.6	1.8; 0.5
F	73.3	78.7	71.7	−5.4; 1.9	1.6; 0.8	7.0; *4.1*
Thoracic index II‖						
M	22.2	20.7	21.9	1.5; *2.7*	0.3; 0.3	−1.2; 1.1
F	21.7	20.4	20.3	1.3; *2.0*	1.4; *2.3*	0.1; 0.2
Umbilical index ¶						
M	103.9	94.3	96.8	9.6; *2.1*	7.1; 0.9	−2.5; 0.4
F	97.7	90.8	97.3	6.9; 1.2	0.4; 0.0	−6.5; 1.5
Arm leg index **						
M	85.4	85.4	83.0	0; 0	2.4; *2.0*	2.4; *2.4*
F	83.5	84.1	84.5	−0.6; 0.7	−1.0; 0.9	−0.4; 0.6
Ponderal index ††						
M	19.5	22.2	21.5	−2.7; *3.6*	−2.0; 1.6	0.7; 0.4
F	21.0	22.6	22.8	−1.6; 0.9	−1.8; 1.2	−0.2; 0.2

M.—male.
F.—female.

* $\dfrac{\text{Nasion prosthion}}{\text{Facial diameter}}$.

† $\dfrac{\text{Nasion submenton}}{\text{Bigonial diameter}}$.

‡ $\dfrac{\text{Neck height}}{\text{Neck circumference}}$.

§ $\dfrac{\text{Thoracic anteroposterior diameter}}{\text{Thoracic lateral diameter}}$.

‖ $\dfrac{\text{Thoracic length}}{\text{Thoracic circumference}}$.

¶ $\dfrac{\text{Umbilicus-pubis}}{\text{Umbilicus-xyphoid}}$.

** $\dfrac{\text{Arm length}}{\text{Leg length}}$.

†† $\dfrac{\text{Weight}}{\text{Height}}$.

the male and female peptic ulcer and cholecystitis groups. Both sets of figures show an increased circumference in the cholecystitis patients. The chest circumference of diabetic males and females also exceeds that of peptic ulcer patients.

In the present series the female peptic ulcer patients showed a larger tibial length than did the cholecystitis patients, whereas Draper's figures tended to show the reverse. Here again the distance measured lay between bony points, so that the absence of similar results suggests that the relationship cannot be a constant nor an important one.

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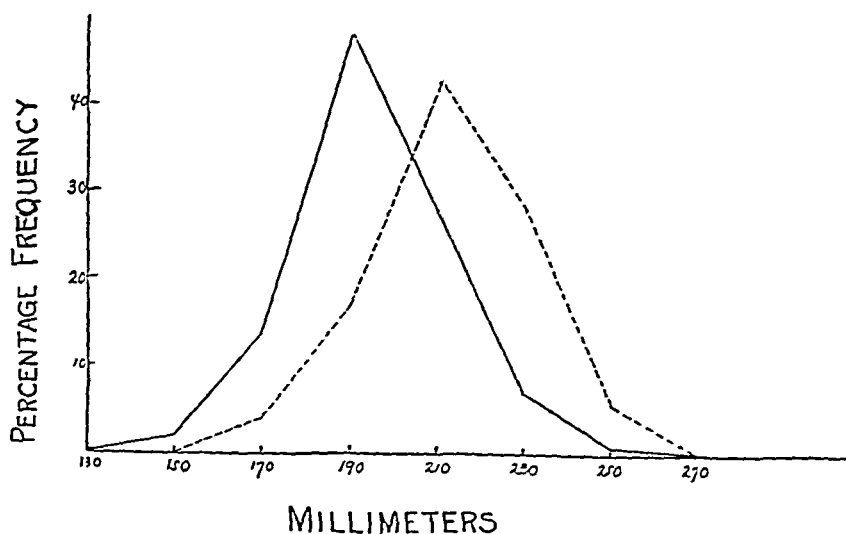
Fig. 5

Thoracic anteroposterior diameter (male). ———— Peptic ulcer cases 55; — — — — Diabetic cases 27. Note the relatively smooth contour of the curves. There is a considerable degree of overlapping, though 't' is 4.5.

The cholecystitis patients in this and Draper's series weighed more than the peptic ulcer cases, a confirmation of an old and a well tried observation. However, it was only in comparing the weight of the male cholecystitis with the male ulcer patients, that the difference was found to be significant.

DISCUSSION

In the last 10 years, many papers have appeared in the literature calling attention to the fact that constitution and disease are interrelated, in the anatomical as well as in the other panels. It is to Draper (5) that we owe the renewed interest in this subject on this continent. Our results do not bear out Draper's conclusions however. The material incorporated in Draper's book "Human Constitution" (5) was drawn from 6 disease groups, namely, peptic ulcer, cholecystitis, pernicious anemia, nephritis with

hypertension, pulmonary tuberculosis, and asthma. The material for the present paper consists of three groups as already seen. Of these, only two were the same as Draper's, and generally, as the following illustrative examples show, our results do not corroborate Draper's conclusions. Thus, the large mandible found by Draper in the male ulcer cases was not present in this series. The dolichocephalic trend did not occur in the ulcer patients of this series, neither did the long, broad ears in the cholecystitis cases. Although the upper facial diameter was slightly longer in the male cholecystitis patients than in the others in both investigations, the difference was too small to be important. The increased neck height of the peptic ulcer race has already been alluded to and was common to both series of patients. The observation that the gallbladder patients show the thickest chests has been confirmed, as has been also the increased chest circumference of these individuals. In a small number of instances then, the trend of the figures in the two series is comparable, but there is in general strikingly little to encourage the student of constitution in the belief that anatomical features are important elements in the etiology of disease. Moreover, a good many students interested in the relation between constitution and disease have been disappointed at the relatively fruitless results of work in this field. Stockard (8) has expressed such an opinion after critically surveying the work which has been done. Udaondo (9), from a wide clinical experience, has concluded that there is no specific type associated with peptic ulcer. Levine, Neal and Park (7), found no relationship between body type in children and predisposition to the development of poliomyelitis. These investigators employed anthropometric methods in 52 patients who had contracted poliomyelitis. These were contrasted with 52 normal controls of the same age and race.

In addition to the material issuing from Draper's laboratory, there have been other contributions supporting his point of view. Barach (3) has described clinical types which he believes are distinct both for males and females, and which are particularly predisposed to the development of hypertension later in life. Anatomical types such as Barach has indicated undoubtedly exist, but it is difficult to link them always with the development of hypertension.

In recent years there has been much speculation as to the importance of constitutional predisposition in the etiology of both exophthalmic goitre and toxic adenoma of the thyroid gland. It has been suggested by many students that of the several factors involved in the production of a toxic goitre, the fundamental one is the individual's constitution. On this basis various anatomical peculiarities have been attributed to the Graves' constitution. Warthin (11) pointed out that Graves' constitution could be recognized histologically by the presence of embryonic lymph follicles in the thyroid tissue. He suggested that these constituted the substratum for the production of goitre, though there must occur coincidentally an altered

functional reaction in the individual. However, so far as can be determined, anthropometric methods have not been applied to the study of constitution in goitre, so that the anatomical type in this disease still remains theoretical.

If constitution is one of the determinants in the incidence of disease, its importance should be as obvious in pediatrics as it is in the diseases of adults. With this in mind the Bakwins (2) studied the body build of infants affected with intestinal intoxication, eczema and tetany as compared with a normal group. In the first of these groups they found that the bigonial diameter and the thoracic circumference were reduced as compared with a normal group. The reverse relationship applied in the series with

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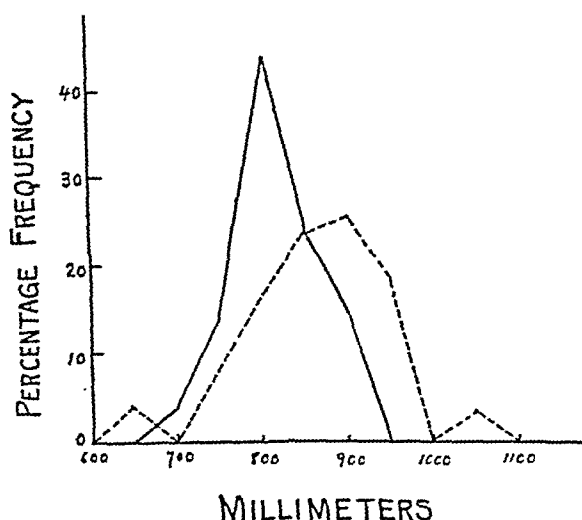
FIG. 6

Chest circumference (male). ———— Peptic ulcer cases 55; — — — —
Diabetic cases 27. Note the disparity between the peak of the diabetic and the peptic ulcer patients. This is borne out by 't' which is 3.6.

eczema and tetany. This work has been carefully done and deserves attention. But the following objection may be raised here. Only four body measurements were compared in the four groups. Therefore one cannot conclude from their results that the relationship between physical types and disease incidence has been proven.

It had been suggested years ago that the size of organs or of systems might determine their predisposition to disease. Brown (4) has attempted to settle this aspect of the problem in the laboratory. He believes that there are two phases of organic constitution which should be considered, namely, the organ-body-weight relation or organ balance, and the organ equilibrium or interrelation of organs. The basic ratio of these states differs in individuals by virtue of heredity. They may vary from month

to month and from year to year because of environmental influences. There is, however, in this connection a normal trend. In summer there is a tendency towards a high positive organ balance due to a predominant endocrine influence (?), while in winter this condition is reversed. During the periods of transition, Brown believes the animal or the human body to be in a state susceptible to disease. While this author has been able to prove the lability of the organ to body and organ to organ relation, he has not been able to prove that these changes are correlated with the development of disease.
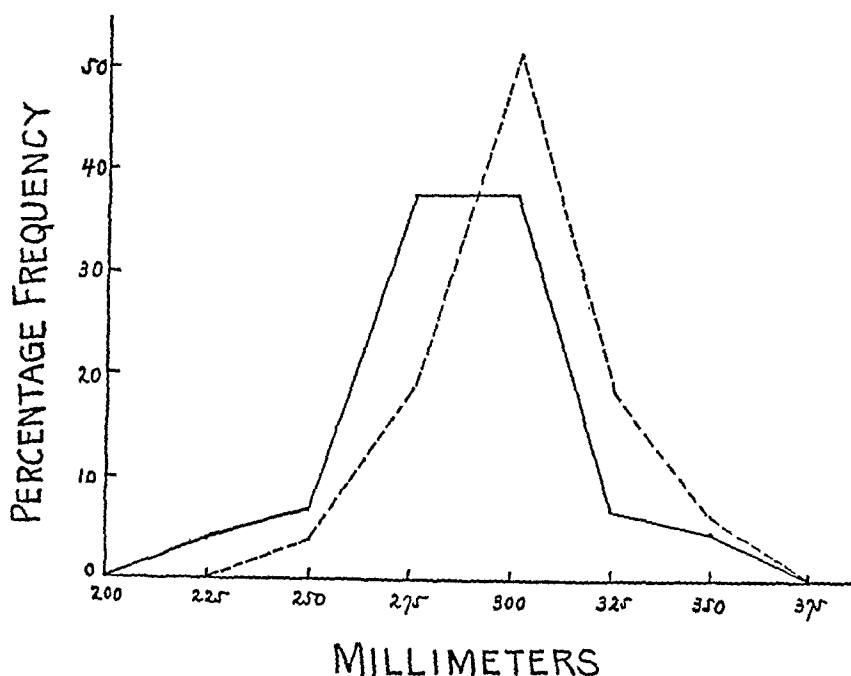
FIG. 7

Leg circumference (male). ———— Peptic ulcer cases 55; — — — — Diabetic cases 27. Note again the flat top of the peptic ulcer group. The area which overlaps in the two curves is rather small.

SUMMARY AND CONCLUSIONS

The question whether physical constitution affects the incidence of disease in the average patient is as old as the scientific study of disease. The evolution of interest in constitution has proceeded along the following lines. Physical form and function were first related to each other by anatomists and physiologists. Then anatomical form and pathological function were linked by the old clinicians and pathologists. However, the only scientific proof for such an association lay in clinical impressions until the recent introduction of anthropometric methods to this problem by di Giovanni (15), Beneke (14), and Draper (5).

The material upon which this paper is based consists of 192 patients in 3 disease groups; 79 patients with diabetes mellitus, 67 with peptic ulcer,

and 46 with cholecystitis. The chief difficulties met with in the work are threefold. In the first place, anatomical features which may be predisposing factors to disease, must be distinguished from the anatomical *effects* of disease upon the body. Secondly, the racial distribution of the patients must be considered. The three groups studied showed practically an equal distribution for the various races. And thirdly, there should be a sufficiently large number of cases in each disease group to make the results of statistical treatment reliable. There was an adequate number in all the groups except in the male cholecystitis and female peptic ulcer patients. In both of the latter, the difficulty produced by small numbers was obviated by suitable mathematical methods. Thirty-seven measurements were taken on each patient, in addition to a record of the condition of the skin and its appendages.

In the final analysis of the results, the value of " t " was obtained and considered. This letter is the ratio between the difference of two means, and the standard error of the difference. When " t " equals or exceeds 2, it is assumed, by statisticians, to be significant. Out of 270 cases in which " t " was obtained, it reached a significant value in 53 instances, or 19 per cent. But, the difference pointed in the same direction for both the male and female patients in only 9 measurements, in the following cases. The bigonial diameter, the thoracic anteroposterior diameter, the thoracic lateral diameter, the thoracic circumference and the thoracic index 1 [2] were all greater in the diabetic than in the peptic ulcer patients. The neck height of the peptic ulcer patients exceeded that of the diabetics. The neck index and the thoracic index II [3] were larger in the peptic ulcer than in the diabetic patients. · The thoracic circumference of the cholecystitis males and females was larger than that of the peptic ulcers. In the remaining cases in which differences were found, they occurred in only one or other of the sexes.

Because of the inconclusive opinions expressed in the literature, and the work presented here, it is thought that, in the past, too much stress has been placed upon the importance of physical constitution in the etiology of disease. At present there is no basis for employing it in diagnosis or in the selection of individuals who will be predisposed to diabetes mellitus, peptic ulcer and cholecystitis.

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[2] $\dfrac{\text{Thoracic anteroposterior diameter}}{\text{Thoracic length}}$

[3] $\dfrac{\text{Thoracic length}}{\text{Thoracic circumference}}$

Professor of Surgery, McGill University, and of his colleagues from whose wards a large number of the patients were drawn. We are indebted to Dr. Edward H. Mason for providing a number of cases from his ward. Miss Helen Stevenson rendered valuable assistance with the anthropologic measurements.

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OBSERVATIONS UPON THE CALCIUM AND PHOSPHORUS METABOLISM IN CERTAIN DISEASES OF BONE

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Until recently practically all studies of the various diseases of bone have concerned only the clinical picture, with occasional investigations of the gross and microscopic anatomy of the diseased tissue. In the literature we have been able to find in the earlier years very few chemical or metabolic studies, but these have been thoroughly done and still form the groundwork of our present knowledge.

With the isolation of the parathyroid hormone and improvements in biochemical methods, renewed interest in the metabolism of calcium has sprung up, part of which has naturally been directed to the processes in bone diseases. The isolation of the clinical entity of hyperparathyroidism as it appears in the long recognized clinical condition of osteitis fibrosa cystica is a triumph for the methods of investigation which combine clinical observation with those made from the pathological and biochemical aspect.

Since the recognition of this new entity there has been the natural desire to make parathyroid dysfunction responsible for other somewhat similar conditions, and Paget's disease of bone (osteitis deformans) has by some been included in this group (1) (2) (3) (4), even to the extent of performing parathyroidectomy in such cases in the hope of curing or improving the condition. However, as yet the evidence in favour of this procedure in these cases is inconclusive and circumstantial.

Recently several studies have appeared which tend to clarify the situation and, as we feel that the number is not yet large enough to impress the fact, we desire to place on record the observations on calcium and phosphorus metabolism which we have made during the course of the past four years in four cases of osteitis deformans, one of solitary bone cyst and one of sarcoma of bone.

GENERAL METHODS

The patients were kept in the special metabolism ward of the hospital, the staff of which has been trained in the collection of complete specimens and the feeding of special diets for observations on metabolism.

The diets were supplied from the special diet kitchen attached to the ward, being taken direct to the patient after they were weighed out; the trays were further checked after each meal to make sure that all food had been eaten.

iliac crests.   There was no obvious deformity of the spine or thighs, and the head was of normal shape and size.   X-rays of the pelvis, skull, both tibiae, and the left femur showed the typical changes of Paget's disease.   In the region of the thyroid, to the right of the midline, and just lateral to the sternal end of the clavicle, was a nodule about the size of an acorn, smooth and firm.   There were no signs of toxic goitre, and the basal metabolic rate varied from plus 24 to plus 18.   There were no other findings of importance.

After the completion of the calcium studies, the nodule in the thyroid region was excised.   Histological examination showed the structure of an adenoma of the thyroid; there was no evidence of parathyroid tissue.   Following its excision the basal metabolic rate was plus 18, and there was no change in the serum calcium.   Owing to accidents in collection and analysis of the specimens, only two complete periods were obtained, the one on low and the other on moderately high calcium intake.   As the patient could not remain longer in hospital no further studies were made.

In February 1933 the patient was seen again when there was no apparent change in his condition either clinically or by x-ray appearance.

Case 4.   Paget's disease, glomerulonephritis and uraemia

Mrs. T., a female, 53 years of age, was admitted on February 5th, 1931, to the medical ward; her main complaints were epigastric pain and vomiting. A further history was obtained of dyspnoea, palpitation, oedema of the ankles, and night frequency with nocturia for the preceding year and a loss of 45 lbs. in weight.

Physical examination.   There was slight anterior bowing of both legs, with enlargement and thickening of the tibiae more marked on the right than on the left.   X-ray showed the typical picture of Paget's disease in both tibiae and fibulae as well as in the femora, vertebrae, pelvis and skull.   X-ray investigation of the gastro-intestinal tract was negative.

The heart was slightly enlarged, the blood vessels were thickened, blood pressure was 170/85.   In the fundi arteriosclerotic changes of the vessels were seen but no haemorrhage or exudate.   The urine specific gravity varied only between 1.010 to 1.015; there was a trace of albumin, and hyaline casts were present.   Nonprotein nitrogen of the blood was 123 mgm. per 100 cc., the creatinine 6.8 mgm. per 100 cc.; serum calcium was 6.8 mgm. per 100 cc. and inorganic phosphorus 5.6 mgm. per 100 cc.

Diagnosis of chronic glomerulonephritis with uraemia and of Paget's disease was made.   As the patient was able after a few weeks to take a sufficient amount of food, studies on calcium metabolism were made under the same regime as the other cases, the balance being determined during three periods of low calcium intake and three periods of moderately high calcium intake.

Case 5.   Solitary cyst of bone

R. T., a six year old boy, was admitted to the surgical service on December 11th, 1929.

In July of 1929 the patient's father first noticed that he was limping in his left leg; shortly after this a swelling was noticed just below the knee which increased slowly in size.   There was no pain or tenderness, and no other symptoms except some loss of weight.

Physical examination on admission (December 1929).   There was swelling just below the left knee joint involving the whole circumference of the leg; it

was hard, firm, and smooth. X-ray showed a solitary cyst of the head of the tibia extending up to, but not invading, the epiphysis.

At operation, December 12th, the cyst was opened and thin blood-stained fluid evacuated. On December 30th the cavity was opened, the walls curetted and the space filled with bone chips from the shaft of the right tibia. Studies of calcium metabolism were made in the following March, at which time the wound was well healed and x-ray showed the presence of bone implants in the cavity and rarefaction of the bones of ankle and knee joints. The other symptoms were negative.

Three periods of observation on low calcium intake were made after which viosterol 250 D. was added in doses of 10 drops three times daily with meals for one period, and increased to 15 drops for the second period. The diet was then changed to one of higher calcium content and after it had become stabilized four periods of observation were carried out.

The patient has recently (July, 1933) been admitted to the Children's Memorial Hospital with another solitary cyst in the right tibia; no further studies of calcium metabolism have been made.

Case 6

Mrs. P., a female, 29 years of age, was admitted on July 6th, 1933, for study of calcium metabolism.

The patient was well until August, 1925, when she "strained" her left forearm while riding a bicycle; however, she was able to continue at her heavy work, shovelling washing powder nine hours daily, until January, 1926. At this time she first noticed a painless swelling of the left forearm just below the elbow; because of its steady increase in size she came to the hospital out-patient clinic one month later. X-ray report at that time suggested a giant cell sarcoma of the upper part of the shaft of the radius: the shaft of the humerus presented an unusual appearance, the medullary portion being greatly rarefied and the cortex thinned out. At operation the tumor tissue was removed from the radius with chisel and curette. The pathologist's diagnosis was "Giant cell sarcoma."

After the operation the patient was given deep x-ray therapy over the radius and humerus at intervals for the next six years. In 1929 she suffered a pathological fracture of the left clavicle which healed well. In the same year she was married, and has since had three pregnancies, both the first two children suffered from spina bifida, the third pregnancy terminated by miscarriage. There has been a gradual loss of weight from 138 to 113 pounds.

On readmission in July, 1933, the physical examination showed telangiectases of the left arm, due to x-ray therapy, and palpable enlargement of the head of the left humerus and of the left elbow, radius, and ulna. In the region of the right lobe of the thyroid there was a nodular mass the size of a walnut, which moved on deglutition and appeared to be fixed in thyroid tissue. There were no other significant physical findings, and the blood Wassermann reaction was negative.

X-ray reports were as follows: "There is a cystic appearance in the left clavicle, humerus, radius, and first metacarpal bone, and a suggestion of slight cystic condition in the right scaphoid. There is no appearance of cystic disease in right and left tibia and fibula, right clavicle, humerus, radius, ulna and metacarpals."

The x-ray pictures of the bones of the left upper extremity were very suggestive of osteitis fibrosa cystica, the mass in the neck suggesting a parathyroid

tumor. However, the normal appearance of the other bones and the normal blood calcium and phosphorus were opposed to this diagnosis. The calcium balance was determined over one period of six days on fixed diet, after which the tumor of the neck was excised. The pathologists's report on the nodule is as follows: " Specimen consists of a slightly lobulated mass about the size of a hen's egg. There is a definite capsule. On cut section it shows a yellowish grey homogeneous surface with narrow fibrous tissue bands dividing it into irregular areas. Numerous sections show the typical microscopic structure of a fetal adenoma of the thyroid which is slightly compressed, otherwise normal, parenchyma. In one area is atypical inclusion of parathyroid cells. This mass is well limited with a poorly defined capsule. The cells have a definite alveolar arrangement and in some places form irregular acini and columns. They are separated by endothelial-lined spaces containing scattered red cells."

DISCUSSION

The figures for the various balances have been condensed into Table I, where the averages for three day periods are shown.

As the cases of osteitis deformans have been the most discussed of late, we shall consider. them together and discuss the other two cases separately. We have also found it more convenient to discuss the calcium and phosphorus metabolism separately.   ·

Calcium

In all four cases of Paget's disease when the low calcium diet (260 to 380 mgm. Ca per diem) was given, the calcium balance was negative and the excretion in the urine was within the limits found in normal individuals on approximately similar calcium intakes (8); it was somewhat low in the fourth case, where the presence of a well marked nephritis produced the usual decrease in urinary calcium (9). Three of these cases were given a higher calcium intake; Case 1 by adding calcium gluconate to the diet, Cases 3 and 4 by feeding a diet high in calcium. The result was to change the negative balance over to the positive side, though in Case 4 where the increased intake was not so high a barely positive balance was reached. The calcium in the urine increased moderately in all, but was negligible in the case complicated with nephritis; the stool content was greater in all cases.

Viosterol was administered in two cases (1 and 2) in relatively large doses. The period over which it was administered, however, was relatively short which may account for the divergent results. In Case 1 it unfortunately followed a period of treatment with parathormone, some effect of which may have been carried over into the viosterol periods. In both cases the amount of calcium excreted in the urine was markedly diminished though that in the stool was increased in Case 1 sufficiently to produce a greater negative balance than in the control period, whereas this negative balance decreased in Case 2. Only in the first case was parathyroid

TABLE I

Calcium and phosphorus metabolism (in the case of diet, urine and feces, figures represent the average for a single period of three days expressed in milligrams)

Case number	Periods Type	Number	Intake	Urine	Stool	Total	Balance	Serum
			mgm. 3 days	mgm. 3 days	mgm. 3 days	mgm. 3 days	mgm. 3 days	mgm. to 100 cc.

Calcium

Case number	Type	Number	Intake	Urine	Stool	Total	Balance	Serum
1. Paget's disease	Control	2	1113	716	598	1314	− 201	10.8
	Parathormone	1	1113	1528	755	2283	−1170	16.9
	Viosterol	2	1113	482	1065	1547	− 434	9.9
	Calcium gluconate	2	3516	964	2244	3208	+ 308	9.8
2. Paget's disease	Control	3	1140	604	1019	1623	− 483	9.5
	Viosterol	2	1140	325	1094	1419	− 279	9.5
3. Paget's disease	Low calcium	1	792	527	765	1292	− 500	8.6
	High calcium	1	3750	893	1690	2583	+1167	8.3
4. Paget's disease, nephritis	Low calcium	3	780	101	1868	1969	−1189	6.9
	High calcium	3	2295	114	2149	2263	+ 32	8.4
5. Solitary bone cyst	Control	3	930	693	701	1394	− 464	10.0
	Viosterol	3	930	696	636	1332	− 402	
	High calcium	4	3039	642	2044	2686	+ 353	10.5
6. Sarcoma of bones	Control	1	930	300	840	1140	− 210	9.8

Phosphorus

Case number	Type	Number	Intake	Urine	Stool	Total	Balance	Serum
1. Paget's disease	Control	2	2271	1799	314	2113	+ 158	3.1
	Parathormone	1	2271	2609	375	2984	− 713	3.7
	Viosterol	2	2271	1455	424	1869	+ 402	
	Calcium gluconate	2	2271	1702	489	2191	+ 80	
2. Paget's disease	Control	3	2232	2695	1102	3797	−1565	3.9
	Viosterol	2	2232	2866	1135	4001	−1769	4.6
3. Paget's disease	Low calcium	1	2160	1738	811	2549	− 389	3.7
	High calcium	1	3900	2098	1062	3162	+ 738	3.7
4. Paget's disease, nephritis	Low calcium	3	1674	649	1547	2196	+ 522	6.4
	High calcium	3	3318	589	1675	2264	+1054	6.4
5. Solitary bone cyst	Control	3	2448	1540	580	2120	+ 328	4.6
	Viosterol	3	2448	1587	505	2092	+ 356	
	High calcium	4	4305	2208	1137	3345	+ 960	4.1
6. Sarcoma of bones	Control	1	1626	1557	597	2154	− 528	3.6

extract used and here it showed the typical effect that would be expected (10) with a doubling of the amount of calcium excreted in the urine and a marked increase of the already negative balance, while the value in the blood serum rose to an abnormal level. In none of these four cases could the calcium excretion be considered to have increased above the normal.

Phosphorus

The balance of phosphorus, unlike that of calcium, varied considerably, with the low calcium diets from decidedly negative figures as in Case 2, to moderately positive as in Case 1. In Case 4 the value also was positive, but this may be related to the renal lesion; it is quite obvious that the excretion in the urine is much smaller here than in the other uncomplicated cases. On the high calcium diet the retention of phosphorus was increased in all but the first case, in which the increase in dietary calcium was made by adding to the diet calcium gluconate; this did not increase the phosphorus intake as it was increased in the other subjects who were given their extra calcium by changes in the food.

The effect of the addition of viosterol was to promote retention in one case and to increase the negative balance in the second. The administration of parathormone caused the usual increased excretion (10), by far the greatest amount being in the urine.

Blood

The level of the calcium in the serum in the uncomplicated cases ranged from 8.3 to 10.8 mgm. per 100 cc. That of 6.9 in the patient with nephritis is within the limits to be expected in this clinical condition (9); it is of interest to note that high calcium diet raised this level to 8.4 mgm. per 100 cc. Parathormone in the one case produced hypercalcemia similar to that observed in normal subjects (10), 16.9 mgm. per 100 cc. being the level obtained. In the cases treated with viosterol no significant change was noted.

The inorganic phosphorus of the serum of the uncomplicated cases was also within normal limits, the high level of 6.4 mgm. per 100 cc. being found, as might be expected, in the case complicated with nephritis (9). In Case 2 there was a moderate rise during viosterol therapy.

These normal levels in the blood again fail to show evidence of parathyroid hyperactivity (9) (10).

Solitary cyst of bone (Case 5)

In this case the calcium and phosphorus balances are approximately those to be found in a normal individual. Here again the balance depends on the intake, while the effect of viosterol is not appreciable unless it be that it promotes a slight increase in absorption and a slight decrease in

excretion. The values of calcium in the serum are normal as are those of the inorganic phosphorus for a child of the age studied. There is absolutely no evidence of increased calcium excretion or changes in the serum to suggest that the condition is due to parathyroid hyperfunction.

Sarcoma of bone (Case 6)

One period only was studied in this case, but this was sufficient to show that the calcium metabolism was essentially normal. The absence of increased calcium or decreased phosphorus in the serum and the excretion of normal amounts of calcium and phosphorus in the urine all argue against any parathyroid hyperfunction, and fit in with the x-ray findings which limit the process to the left forelimb.

COMMENT

In spite of the large and ever-increasing literature on the subject of bone disease we have been able to find only a few studies of the calcium metabolism in Paget's disease, and of these several do not stand critical examination. The extreme importance of the accurate time collections of specimens, of careful dietary supervision, of the use of reliable technical methods, would seem to need no emphasis, yet in many cases discrepancies in results are undoubtedly due to avoidable errors in these factors. A surprising number of authors merely state their results, with no details of technical procedures.

The case reported by Gruner, Scrimger and Foster (11) in 1912 showed a negative balance of calcium on an intake of 1019 mgm. daily. Although the stools were apparently properly marked off and collected for the single period of study, yet the analytical results imply that calcium was determined in the urine only, the calcium for feces being left blank. As the " total outgo," however, corresponds with the amount in the urine, there is the possibility that urine and feces may have been analyzed together. If the figure actually represents urine only, there is undoubtedly a marked increase in the calcium, similar to that seen in hyperparathyroidism. However, as the patient later died with diffuse sarcomatous changes in the bones, which were apparently active at the time of the metabolic studies, this excess excretion may have been due to the excessive destruction of bone.

In 1914 Da Costa, Funk, Bergeim and Hawk (12) reported observations on two cases of Paget's disease which were thoroughly studied. In both cases they found a positive calcium balance with intakes of approximately 1 gram and 1.6 gram of calcium respectively daily. The urinary excretion was not greater than could be considered normal, and in the more advanced case tended to run slightly low, approaching the level found in our Case 4, though in their protocol there is no mention of renal disease. The positive balance was greater in this case, which led the authors to

suggest that in the more advanced case there is a greater storage of calcium, while in the earlier case the excretion is greater. From the sulfur balance, which was in equilibrium in the early case, negative in the advanced, they suggested that the early case was laying down an organic matrix which was displaced in the later case by deposits of calcium to form bone. This view has remained in the literature and still appears from time to time. The phosphorus balance was also positive, as was that of magnesium.

In 1927 Cuthbertson (13) reported findings in a single carefully studied case. He also found a positive balance with a daily intake of calcium of approximately 2 grams; the excretion in the urine was within normal limits, the level in the blood serum 11.3 mgm. per 100 cc., which he calls a normal figure. The phosphorus and magnesium balances were also positive; that of sulphur was negative. From his figures he suggests that the new tissue being laid down is relatively richer in magnesium and calcium and poorer in phosphorus than is normal bone.

In the same year a single case of Paget's disease was studied over a period of ten days by Van Hazel and Andrews (14). On a milk and water diet, the calcium balance was decidedly positive, with an intake of 1.245 gram and an excretion of 0.345 gram daily, of which the urine accounted for 0.141 gram, a value quite within normal limits. The calcium in the blood, however, was found to be 13.05 mgm. per 100 cc., though it was " lower " when analysis was repeated at the end of the period. No figures for phosphorus and no details of the technic employed in the calcium studies are given.

Six cases of Paget's disease were reported by Snapper (15) in 1931. He does not state the actual calcium intake but gives the composition of the diet, the calcium content of which we have estimated approximately at 0.250 gram daily. On this low intake the balance is definitely negative, but the amounts in the urine are within the limits excreted by normal subjects on similar diets and much smaller than those observed in a case of hyperparathyroidism studied similarly. He states that the same was true of the phosphorus excretion but gives no figures. The levels of both calcium and phosphorus in the blood fall within normal limits in all the cases.

In a paper in which there is no reference to methods of study or technic of analysis employed, Langeron, Paget and Cordonniere (3) in 1932, reported one case in which, with a daily intake of approximately 0.423 gram, the balance was negative. The urinary excretion was again within the limits found by other workers. After a course of treatment for one month, including Roentgen therapy of parathyroids and suprarenals, and a higher calcium intake, together with irradiated ergosterol, the balance was again determined on an intake of 0.307 gram daily and again found negative, though less markedly so. The levels of calcium in the serum varied from 8.3 to 9.8 mgm. per 100 cc. No phosphorus figures are given

except for serum, 3.21 mgm. per 100 cc. The authors, apparently ignoring the low calcium intake, draw attention to the negative calcium balance in the presence of a normal serum calcium and suggest that this is due to parathyroid hyperfunction in spite of the failure to find an increase in urinary or serum calcium. They, therefore, because of this negative balance, approve of parathyroidectomy as a means of therapy but only on theoretical grounds.

In the week following this communication, Laederich, Mamou and Beauchesne (16) reported in opposition a case in which, with a calcium intake of 2.178 grams daily, the balance was positive, the excretion in the urine low and the level in the serum 9.6 mgm. per 100 cc. The phosphorus balance, on the other hand, was negative with an approximately normal amount in the urine, and 3.9 mgm. per 100 cc. in the blood. No reference is given for analytical technics, and there is a possibility that the collection of feces was incomplete with the methods employed. They conclude that the condition can have no relation to parathyroid hyperfunction.

Labbé with his collaborators (17) has reported studies on four cases in which, with daily intakes ranging from 1.288 to 3.374 grams of calcium, the balances are all positive and in no case is the excretion in the urine increased; in the second case it is extremely low. The calcium in the serum reported in three of the cases ranges from 9.7 to 11.4 mgm. per 100 cc. Phosphorus balances, reported in only two cases, were negative in both. In the blood the values were 2.7 and 4.8 mgm. per 100 cc. Normal control cases gave positive balances for both elements. The authors do not state the methods used in the collection of specimens but describe their analytical procedures. A feature difficult to understand is that though the four patients each received two litres of milk daily as "alimentation unique," yet, the calcium intake of each differed, in some cases to such an extent as to be beyond the margin of error allowed for analytical procedures.

A single case was carefully studied by Rabinowitch (18), who found that with calcium intakes of 2.250 grams and 2.450 grams daily the balance was positive. The excretion in the urine corresponded with the average case in the literature and the serum calcium was 9.2 mgm. per 100 cc. Phosphorus balance was also positive with an intake corresponding closely to that of calcium; the serum inorganic phosphorus was 3.8 mgm. per 100 cc. Like Da Costa, Funk, Bergeim and Hawk (12) and Cuthbertson (13), he also found a negative sulphur balance and a storage of magnesium. After a regime of quartz light therapy there was relatively little change. He agrees with Bergeim and Hawk that the calcium, magnesium and phosphorus balance depends on the stage, severity and extent of the disease, and states that there is no relation between the quantitative intake of calcium and the retention.

Recently Bauer (19) has reported summaries of seven cases of Paget's disease, in all of whom the blood findings were normal and in two of

whom there was a slight increase of calcium and phosphorus excretion, not confined solely to the urine. His paper is an excellent argument against there being any element of hyperparathyroidism in Paget's disease.

SUMMARY OF THE LITERATURE

In Table II we have summarized the calcium and phosphorus balances found in the literature, arranging them according to ascending value of

TABLE II

Summary of calcium and phosphorus metabolism data found in the literature (values represent the average for a single day expressed in milligrams of Ca and P (in the serum as mgm. per 100 cc.))

Author	Number of cases†	Intake	Output			Balance	Serum
			Urine	Stool	Total		
Calcium							
		mgm. per day	mgm. per day	mgm. per day	mgm. per day	mgm. per day	mgm. per 100 cc.
Snapper (15)........	7	250*	74	348	533	−283	9.9
			to	to	to	to	to
			207	968	1089	−739	11.6
Langeron (3).......	1	⌈ 423	295	354	649	−226	8.3
		{					to
		⌊ 307	75	330	405	− 98	9.8
Gruner (11)........	1	1019	1477?		1477	−458	
Bergeim (12).......	1 (M)	1141	16	551	567	+574	
Van Hazel (14)......	1	1245	140	250	390	+855	13.05 and "lower"
Labbé (17).........	1 (R)	1288	140	363	503	+785	
Bergeim (12)........	1 (W)	1846	84	1429	1513	+333	
Cuthbertson (13)....	1	2034	119	1615	1734	+300	11.4
Labbé (17).........	1 (V)	2077	142	1621	1763	+314	9.7
Laederich (16)......	1	2178	54	1379	1433	+745	9.6
Labbé (17).........	1 (C)	2273	14	1639	1653	+620	8.8
Rabinowitch (18)....	1	⌈2250	283	1010	1293	+957	9.2
		⌊2450	373	1092	1465	+985	
Labbé (17).........	1 (D)	3374	87	1156	1243	+2131	11.4
Phosphorus							
Bergeim (12)........	1 (M)	1711	613	527	1140	+571	
Labbé (17).........	1 (C)	1808	540	2671	3211	−1403	2.7
Labbé (17).........	1 (V)	1822	1249	1040	2289	−467	4.0
Bergeim (12)........	1 (W)	1852	749	573	1322	+530	
Cuthbertson (13)....	1	1872	771	1012	1783	+ 89	
Laederich (16)......	1	1881	848	1514	2362	−481	3.9
Rabinowitch (18)....	1	⌈2100	1133	608	1741	+359	3.8
		⌊2200	958	443	1401	+799	

* Figured approximately, actual value not stated by the author.

† The letters after the number of cases indicate the particular subject in a series.

intake. It will be noted that in the case of calcium there is a sharp dividing line between the positive and the negative group, corresponding with an approximate intake of one gram of calcium daily, those cases receiving less than this being in negative balance, those receiving more, in positive balance. The excretion of calcium in the urine is apparently not influenced by the calcium intake, and falls within normal limits. The single case of Gruner (11) stands on the dividing line; the large excretion of calcium in the urine may be due either to analytical error or to a diffuse sarcomatosis affecting the bones.

Serum calcium is within the limits accepted by the individual authors as normal for the analytical procedure employed, with the exception of Van Hazel's case (14), where it was found "lower" when the analysis was repeated.

Phosphorus balances are reported in only seven of these cases, the intake ranging from 1.711 to 2.200 grams daily. Of these seven, four were in positive phosphorus balance, three on the negative side; all seven received a high calcium and correspondingly high phosphorus intake. The "blood" phosphorus when reported was within normal limits.

CONCLUSIONS

In four cases of Paget's disease and one of solitary cyst of bone, when a low calcium intake was given deliberately the balance was definitely negative, as in normal individuals (20). When the calcium intake of four of these same subjects was increased to one gram or more daily (approximately the intake in the case of sarcoma also) the balance swung to the positive side, whether the calcium was administered in the form of foodstuffs, or added to the diet as calcium gluconate. The phosphorus balance was similar to that of the calcium, in that increase in phosphorus intake tended to increase the retention. In the one case where calcium gluconate was added to the diet, with no corresponding increase of phosphorus intake, there was no increase in phosphorus retention, but a slight decrease. This would suggest that the variations in phosphorus balance with increase in dietary calcium depend upon a corresponding increase in dietary phosphorus and not on the calcium intake alone.

In view of the fact here established that the balance depends on the intake, a fact confirmed by our analysis of the literature, it is of interest to consider whether the idea which is expressed in the literature that Paget's disease in its early stages shows increased calcium excretion and in its later stages a calcium storage, might not actually be dependent on the calcium intake in these cases. Rabinowitch's patient (18), according to the history, was relatively early in the disease when studied yet showed a marked retention of calcium, even greater than those of Da Costa, Funk, Bergeim and Hawk (12) who, however, had lower intakes of calcium and had suffered from the disease process for much longer periods. As

none of our cases could be described as early, our results are applicable only to the late stage of the disease.

Our cases, in common with the others reported, fail to show evidence of the increased calcium excretion in the urine or of the increased calcium or decreased phosphorus in the serum which is so typical of the hyperparathyroid syndrome as seen in osteitis fibrosa cystica (19). As far as one is able to study it, the general calcium metabolism is normal.

The administration of parathyroid extract in one case gave the same results as in normal subjects, a result hardly to be expected had the patient *previously* been the subject of parathyroid hypersecretion to which he had developed a resistance similar to that induced in rats by Selye (21).

In one case of Paget's disease complicated with advanced nephritis, the calcium and phosphorus metabolism showed the usual marked decreased excretion in the urine with the lowering of the calcium and increase of inorganic phosphorus in the serum found in this renal condition.

It is of clinical importance that two of these cases of bone diseases had palpable nodules in the thyroid region which at once suggested parathyroid tumours. This was especially so in Case 6, where the x-rays of the affected bones had a marked similarity in appearance to those found in parathyroid hyperfunction. However, the results of the metabolism investigation failed to support this idea, and the report of the pathologist on the excised nodules in Case 3 showed only thyroid tissue; in Case 6, in addition to thyroid tissue which made up the greatest part of the cyst, an area of parathyroid cells was found, but these gave the appearance of " inclusions " not uncommonly found in thyroid tissue, and did not suggest a tumour growth or hyperplasia.

SUMMARY

In four cases of Paget's disease, one of solitary bone cyst and one of sarcoma of bone, the calcium and phosphorus metabolism was found to run within normal limits. No chemical evidence was found to link these cases with parathyroid dysfunction.

Our studies and an analysis of the literature show that a calcium intake of at least one gram daily is necessary to keep these patients in positive calcium balance.

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ANTISTREPTOLYSIN CONTENT OF THE BLOOD SERUM IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS

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Generally speaking immunity to streptococcal infection is slight and of short duration. One of the reasons for this is the great invasive powers of the streptococci, and the irregularity with which demonstrable antibodies are formed in patients with streptococcal infection. Immune bodies can, however, be demonstrated in some patients following streptococcal infection, and attempts have been made to show that there are several types, which may be divided into three groups: antibacterial, antitoxic, and allergic.

Antibacterial immunity is the result of the body's response to the streptococci themselves, and the presence of such antibodies is demonstrated by finding antistreptococcal agglutinins or precipitins in the blood serum. Using these methods, Schlesinger and Signy (1), and Coburn and Pauli (2), have shown that immunity of this type can be demonstrated for a very short time following infection, and, indeed, we have found in common with others that agglutinins may be formed very irregularly following streptococcal infection. It should be mentioned here that caution must be used in interpreting the significance of streptococcal agglutinins unless the precautions, as outlined by Tillett and Abernethy (3), are followed. ,

Antitoxic immunity depends, not only upon the response of the individual, but also on the nature and amount of toxic substances or toxins elaborated by streptococci. It is recognized that toxins vary in their specificity and many streptococci either produce very little toxin, or none that can be demonstrated. The Dick test and the neutralization of Dick toxin have offered methods of demonstrating antitoxic immunity, and although the technique is somewhat difficult and lacking in precision, its employment has provided useful information.

Allergic reactions to the products of the growth of the streptococci, as demonstrated by skin tests to the various chemical fractions, such as the nucleoprotein or toxic filtrates, have yielded information regarding the incidence of streptococcic infection in various conditions, and have provided another method for the study of immune reactions in such patients.

Aside from these methods for studying immunity, Todd (4) (5) has recently called attention to a substance, antistreptolysin, which is present in the blood serum and is capable of neutralizing the hemolytic substance formed *in vitro* by hemolytic streptococci. He has found that following hemolytic streptococcal infections, this substance increases in amount in the blood serum. This method then, offers another means of investigating the response of the body to hemolytic streptococcal infection.

In previous papers by Keefer, Myers and Oppel (6), the presence of agglutinins and skin reactions to the nucleoprotein fraction of *Streptococcus scarlatinae* were studied in patients with rheumatic fever and rheumatoid arthritis, and in a group of miscellaneous hospital patients in an attempt to determine the relationship, if any, between streptococcal infection and these diseases. In pursuing the investigation further, we have studied the antistreptolysin content of the blood serum of two hundred and twenty patients at different times during the course of their disease. The types of disease these patients had are summarized in Table I and Figure 1.

In addition to determining the antistreptolysin titre of the serum, we also studied the agglutination reaction of the blood serum to the same

TABLE I

Antistreptolysin titre of human sera

	Number of patients	Number of determinations	Maximum antistreptolysin titre	Minimum antistreptolysin titre	Average antistreptolysin titre	Number of determinations above average	Per cent of determinations above average	Number of determinations above 200 units per cc.	Per cent of determinations above 200 units per cc.
			units per cc.	*units per cc.*	*units per cc.*	*units per cc.*	*per cent*		*per cent*
Normal individuals.....	20	73	500	30	213	27	37.0	27	37.0
Scarlet fever (Convalescent).....	71	71	5000	100	752	29	40.8	63	88.7
Erysipelas...........	18	64	3000	50	598	17	26.6	42	65.6
Acute respiratory infections (hemolytic streptococcus).....	19	31	800	40	356	14	45.2	22	71.0
Miscellaneous streptococcal infections...	11	17	1000	40	300	6	34.5	6	34.5
Infections not caused by streptococcus......	14	29	500	50	210	10	34.4	10	34.4
Rheumatic fever.......	33	204	2000	100	512	67	32.8	160	78.4
Gonorrheal arthritis....	16	30	500	50	208	10	33.3	10	33.3
Miscellaneous types of arthritis..........	5	12	300	50	105	1	8.3	1	8.3
Rheumatoid (atrophic) arthritis..........	13	61	800	20	196	14	23.0	14	23.0
TOTAL........	220	592	5000	20					
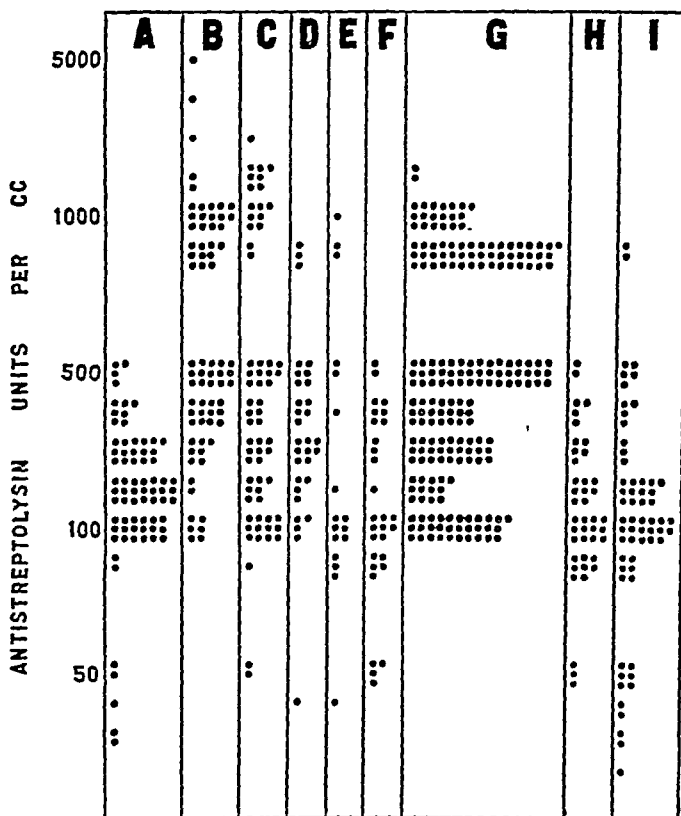
FIG. 1. ANTISTREPTOLYSIN TITRE OF HUMAN SERA.

Each dot represents one determination—*A*, Normal individuals; *B*, Convalescent scarlet fever patients; *C*, Erysipelas patients; *D*, Patients with acute streptococcal respiratory infections; *E*, Patients with other infections caused by streptococci; *F*, Patients with infections *not* caused by streptococci; *G*, Rheumatic fever patients; *H*, Patients with rheumatoid arthritis; *I*, Patients with gonorrheal arthritis and miscellaneous forms of arthritis.

organism from which the hemolysin had been made. In a number, skin reactions to the nucleoprotein of the hemolytic streptococcus and to Dick toxin were tested in the same individuals.

METHODS OF STUDY

All patients were studied while being kept in the hospital. Blood was tested for its antistreptolysin content at different stages of the disease process, using the following technique.

Streptococcal hemolysin was prepared by a modification of the method described by Todd (4). Veal muscle infusion broth containing 2 per cent Difco proteose-peptone and 0.5 per cent sodium chloride adjusted to a pH of 8.0 was used. To this medium was added yeast extract (7 cc. per 100

cc.) prepared from Mead's Powdered Brewers' Yeast. The mash inoculated from a six-hour culture of a hemolytic streptococcus (NY and incubated for sixteen hours at 37° C. At first the culture was incubated under a vaseline seal but it was found that the yield of strept. was not enhanced by the anaerobic culture. The culture was centrifuged at high speed for 45 minutes to remove the organisms. The supernatant fluid was treated with 0.5 gram of sodium hydrosulphite and 1.0 cc. normal sodium hydroxide to each 100 cc. The air was withdrawn by means of a water suction-pump for 30 minutes. The pH of the solution was then adjusted to 7.6 or 7.7 and the solution stored under oil in tubes in the icebox. The streptolysin used was of such potency that 0.2 cc. would completely hemolyze 0.5 cc. of a 5 per cent suspension of washed sheep cells in normal saline in conformity with the procedure of Todd (4).

The titration of the antistreptolysin of the sera was carried out after the sera had been inactivated for fifteen minutes at 56° C. Dilutions of the serum in normal saline were prepared. The dilutions used were 1:20, 1:30, etc. to 1:4000 and 1:5000. To 1.0 cc. of each dilution was added 0.5 cc. of streptolysin solution. This was shaken and incubated for 30 minutes at 37° C. One-half cc. of a 5 per cent suspension of washed sheep red blood cells in normal saline was added. The tubes were then shaken and incubated for one hour at 37° C., being shaken again fifteen minutes after the addition of the sheep cells.

The titre of antistreptolysin of the serum tested is expressed in units as the reciprocal of the fraction of one cc. of serum just sufficient to inactivate 0.5 cc. of the streptolysin solution (Todd (5)). A unit of antistreptolysin may be said to be the amount necessary to inactivate 0.5 cc. of streptolysin solution of the potency prescribed above. For example a given serum is said to contain twenty units per cc. when one cc. of the 1:20 dilution is the least amount necessary to inactivate 0.5 cc. of standard streptolysin solution.

At the time of each determination a horse serum of known antistreptolysin titre was used as a control. This was necessary in order to ascertain that the hemolysin was constant and potent.

RESULTS

There were made 592 determinations of the antistreptolysin titre of the blood sera from 220 individuals. Their ages varied from 5 to 84 years. It was not possible to show any correlation between the age of the individual and the titre of the antistreptolysin of the blood serum.

As controls, we studied normal individuals without streptococcal infection, horse serum obtained from horses that had been immunized against the streptococcus, and horse serum from horses immunized against other antigens.

The normal individuals whose sera were studied were laboratory workers who did not present clinical features of any streptococcal infection during the period of observation. Two individuals were followed biweekly for 4 months. The blood sera of the other 18 normal individuals were studied every 4 weeks for a period of from 2 to 4 months. The results are summarized in Table I and Figure 1. Sera from the same individual varied from determination to determination, occasionally from one dilution to the adjacent one and rarely to the second dilution. In Table II this is demonstrated in 4 individuals.

TABLE II

Variation in antistreptolysin titres in four normal individuals over a period of five months

Individual	Antistreptolysin titres—Units per cc.				
	Months				
	1	2	3	4	5
1	100		100	100	200
2	30	30	50	40	
3	200		300	200	300
4	100		100	100	100

In order to determine the differences between the antistreptolysin titre of the blood serum of horses immunized with hemolytic streptococci, and those immunized with other organisms, 16 specimens of blood serum were studied. The results are summarized in Table III.

It is seen that there are wide variations in both groups, but it is clear that the sera of horses immunized with streptococci showed a much higher titre with one exception than the sera from non-immunized animals. This

TABLE III

Antistreptolysin titre of horse serum

Specimen	Serum from horses immunized with hemolytic streptococci	Specimen	Serum from horses immunized with other organisms
	units per cc.		*units per cc.*
1.	1,000	7.	5,000
2.	1,000	8.	200
3.	6,000	9.	100
4.	10,000	10.	80
5.	10,000	11.	60
6.	50,000	12.	30
		13.	30
		14.	20
		15.	10
		16.	10

cc.) prepared from Mead's Powdered Brewers' Yeast. The media was inoculated from a six-hour culture of a hemolytic streptococcus (NY5) and incubated for sixteen hours at 37° C. At first the culture was incubated under a vaseline seal but it was found that the yield of streptolysin was not enhanced by the anaerobic culture. The culture was centrifuged at high speed for 45 minutes to remove the organisms. The supernatant fluid was treated with 0.5 gram of sodium hydrosulphite and 1.0 cc. of normal sodium hydroxide to each 100 cc. The air was withdrawn by means of a water suction-pump for 30 minutes. The pH of the solution was then adjusted to 7.6 or 7.7 and the solution stored under vaseline in tubes in the icebox. The streptolysin used was of such potency that 0.2 cc. would completely hemolyze 0.5 cc. of a 5 per cent suspension of washed sheep cells in normal saline in conformity with the procedure of Todd (4).

The titration of the antistreptolysin of the sera was carried out after the sera had been inactivated for fifteen minutes at 56° C. Dilutions of the serum in normal saline were prepared. The dilutions used were 1 : 20, 1 : 30, etc. to 1 : 4000 and 1 : 5000. To 1.0 cc. of each dilution was added 0.5 cc. of streptolysin solution. This was shaken and incubated for 30 minutes at 37° C. One-half cc. of a 5 per cent suspension of washed sheep red blood cells in normal saline was added. The tubes were then shaken and incubated for one hour at 37° C., being shaken again fifteen minutes after the addition of the sheep cells.

The titre of antistreptolysin of the serum tested is expressed in units as the reciprocal of the fraction of one cc. of serum just sufficient to inactivate 0.5 cc. of the streptolysin solution (Todd (5)). A unit of antistreptolysin may be said to be the amount necessary to inactivate 0.5 cc. of streptolysin solution of the potency prescribed above. For example, a given serum is said to contain twenty units per cc. when one cc. of the 1 : 20 dilution is the least amount necessary to inactivate 0.5 cc. of standard streptolysin solution.

At the time of each determination a horse serum of known antistreptolysin titre was used as a control. This was necessary in order to ascertain that the hemolysin was constant and potent.

<center>RESULTS</center>

There were made 592 determinations of the antistreptolysin titre of the blood sera from 220 individuals. Their ages varied from 5 to 84 years. It was not possible to show any correlation between the age of the individual and the titre of the antistreptolysin of the blood serum.

As controls, we studied normal individuals without streptococcal infection, horse serum obtained from horses that had been immunized against the streptococcus, and horse serum from horses immunized against other antigens.

The normal individuals whose sera were studied were laboratory workers who did not present clinical features of any streptococcal infection during the period of observation. Two individuals were followed biweekly for 4 months. The blood sera of the other 18 normal individuals were studied every 4 weeks for a period of from 2 to 4 months. The results are summarized in Table I and Figure 1. Sera from the same individual varied from determination to determination, occasionally from one dilution to the adjacent one and rarely to the second dilution. In Table II this is demonstrated in 4 individuals.

TABLE II

Variation in antistreptolysin titres in four normal individuals over a period of five months

Individual	Antistreptolysin titres—Units per cc.				
	Months				
	1	2	3	4	5
1	100		100	100	200
2	30	30	50	40	
3	200		300	200	300
4	100		100	100	100

In order to determine the differences between the antistreptolysin titre of the blood serum of horses immunized with hemolytic streptococci, and those immunized with other organisms, 16 specimens of blood serum were studied. The results are summarized in Table III.

It is seen that there are wide variations in both groups, but it is clear that the sera of horses immunized with streptococci showed a much higher titre with one exception than the sera from non-immunized animals. This

TABLE III

Antistreptolysin titre of horse serum

Specimen	Serum from horses immunized with hemolytic streptococci	Specimen	Serum from horses immunized with other organisms
	units per cc.		units per cc.
1.	1,000	7.	5,000
2.	1,000	8.	200
3.	6,000	9.	100
4.	10,000	10.	80
5.	10,000	11.	60
6.	50,000	12.	30
		13.	30
		14.	20
		15.	10
		16.	10

seems to be ample proof that antistreptolysins appear in the blood serum following streptococcal infection, and is in accord with the previous observations of Todd (4) (5).

Seventy-one specimens of blood sera were obtained during convalescence from scarlet fever patients approximately 4 weeks after the onset of the disease. It was found that the titre of the serum, as far as the antistreptolysin content was concerned, varied between 100 and 5000 units per cc.—a wide range. On the average, however, the titre was much higher than for the normals, or in the other streptococcal infections studied. Six patients had received human convalescent scarlet fever serum (30 to 55 cc.). The titre of antistreptolysin in the serum of these patients was 300, 400, 400, 500, 500, and 800 units per cc.

The blood sera from 18 patients with erysipelas were studied during the course of their disease. The results are summarized in Table I and Figure 1. The titre varied according to the stage of the disease in which
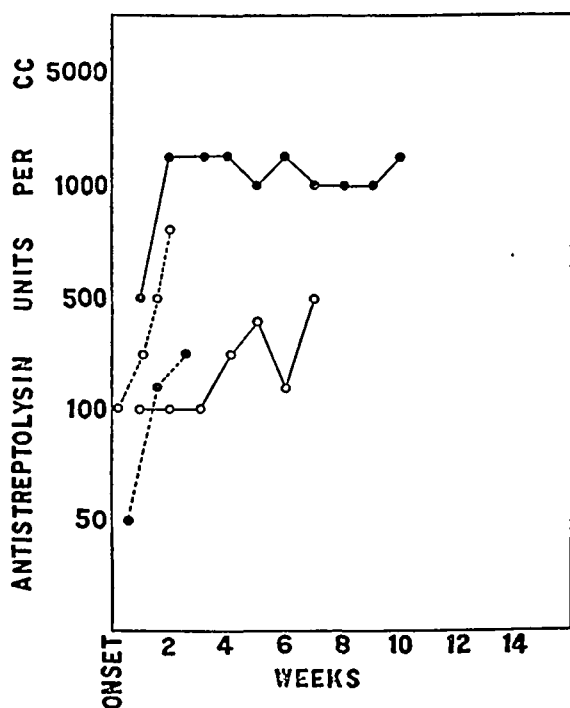
FIG. 2. THE CHANGES IN ANTISTREPTOLYSIN CONTENT OF THE SERA FROM FOUR PATIENTS WITH ERYSIPELAS.

the determinations were made, and in different patients. Eight patients received anti-erysipelas serum, containing a high titre of antistreptolysin, early in the disease. In Table IV the titres of antistreptolysin in the sera of these patients during convalescence are compared with the antistreptolysin content of 8 patients during convalescence similarly treated except that no antisera were given. It is evident that the administration of serum

Antistreptolysin titres of serum from patients convalescing from erysipelas

Specimen	Treated with antisera	Specimen	No serum therapy
	units per cc.		*units per cc.*
1.	200	9.	3000
2.	800	10.	2000
3.	500	11.	1000
4.	400	12.	500
5.	400	13.	400
6.	300	14.	400
7.	300	15.	200
8.	200	16.	100

did not influence the amount of this antibody formed as a result of the infection.

The usual course of events was to find normal or slightly increased titres at the onset of erysipelas and a gradual rise, which was maintained for some weeks. The increase in antistreptolysin titre of the sera of patients during the course of an attack of erysipelas is illustrated in Figure 2.

Acute follicular tonsillitis due to hemolytic streptococci infection was present in 19 patients whose blood serum was examined. The titre varied in individual cases from 40 to 800 units per cc. In some cases an increase in the titre was observed as the disease progressed; in others, this was not apparent.

Of the cases listed in Table I as miscellaneous streptococcal infections, there were 7 of puerperal sepsis and bacteremia, 2 of subacute bacterial endocarditis, all with a'-streptococci in their blood stream. One patient had a β-hemolytic streptococcus empyema with bacteremia, and one had acute hemorrhagic nephritis with hemolytic streptococci in the throat. In these cases, there was a wide range in the titre of the blood serum, 40 to 1000 units per cc. This was true in spite of the fact that in a number of these patients the infections had been present for from 4 to 6 weeks.

It is seen then, that patients with infections due to the hemolytic streptococcus frequently develop antistreptolysins of a high titre in their blood serum. There are wide variations, and the titre may never exceed that which is found in many individuals without streptococcal infection. The highest titres were observed in patients with scarlet fever and erysipelas; somewhat lower titres occurred in acute follicular tonsillitis and in miscellaneous streptococcal infections. It was true, moreover, that the average titre of the blood serum was higher in these groups of patients than in normal individuals.

In order to compare the titre of the blood serum from patients with proven streptococcal infections, with that from patients with other infec-

tions, such as, pneumococcus, staphylococcus, and tubercle bacillus infection, 29 determinations were made on 14 patients with these latter conditions. The titre varied from 50 to 500 units per cc. In other words, it was on the average lower than in proven cases of streptococcal infection.

After demonstrating that the average antistreptolysin content of the serum of patients with proven streptococcal infections was higher than in normal individuals, or in patients with other types of infection, the blood serum from patients with rheumatic fever and rheumatoid, gonococcal, and other forms of arthritis was studied in the same way. The results are summarized in Table I and Figure 1.

In selecting cases of rheumatic fever, we chose only cases in which active infection could be demonstrated either by the clinical course, as characterized by the fever, or by endocarditis, subcutaneous fibroid nodules, or electrocardiographic changes. Thirty-three cases were studied; 18 were followed for 6 weeks or longer at weekly intervals, so that the variation in the antistreptolysin titre could be observed. The titre varied from 100 to 2000 units per cc., and the variation from week to week is illustrated in Figure 3. There were observed three general types of reaction: 1) the
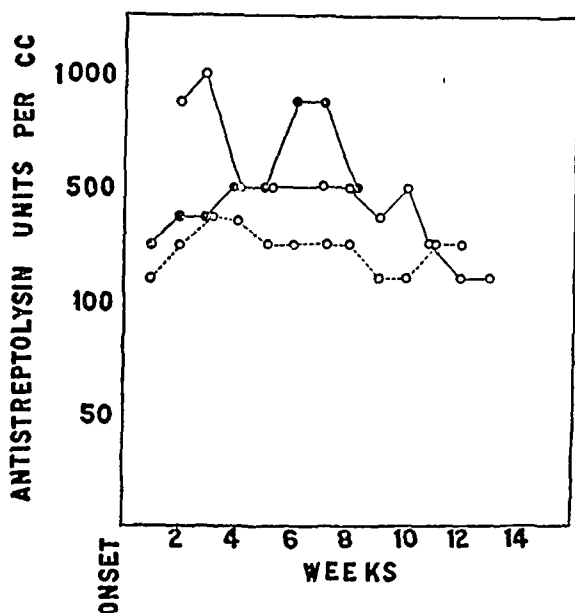
FIG. 3. THE CHANGES IN ANTISTREPTOLYSIN TITRES OF SERUM FROM THREE
PATIENTS WITH RHEUMATIC FEVER.

antistreptolysin titre was higher on the first or second examination than it was on subsequent examinations, and there was a tendency for the titre to decrease from week to week, although it sometimes remained elevated for at least 10 weeks; 2) the titre was low at first and increased and remained elevated with slight fluctuation during the period of observation; and, 3) little change occurred in the titre from week to week, and the

titre never became greater than in some normal people during a similar period of time. Indeed, in 7 of the 33 patients the antistreptolysin titre was never found to be higher than 200 units per cc. It is clear, however, that the average titre of the cases with rheumatic fever was greater than in normal individuals, and was about the same as for cases with proven streptococcal infections. This is not surprising. Inasmuch as rheumatic fever is frequently preceded by a streptococcal infection, one would expect to find a higher titre in patients who develop rheumatic fever following streptococcal infection than in those without a preceding streptococcal infection.

In the 13 cases of rheumatoid arthritis, 61 determinations were made. We selected only cases showing evidence of an active process. In most of these patients the disease had existed for many months, and there was no recent history of an acute streptococcal infection. The range of the titre of the blood serum was considerable; but on the whole, the titre was low, and the average was not greater than in normal individuals. In no case did the titre increase during observation. Thus, the findings were quite different from those in patients with proven streptococcal infections.

In 13 cases of gonococcal arthritis and in 5 cases of miscellaneous types of joint infection the titres were similar to those of rheumatoid arthritis. They were well within the normal range.

It is clear then, that the antistreptolysin content of the blood serum of patients with rheumatic fever, who have had a streptococcal infection, was comparable with the findings in patients with proven hemolytic streptococcal infection but without rheumatic fever. The titre of the sera of patients with rheumatoid, as well as other forms of arthritis, fell into a group which resembled that of normal individuals or patients with non-streptococcal infections.

The relationship between the antistreptolysin titre and the agglutination reaction

We have shown in previous work (6) that there is no direct correlation between the presence of streptococcal agglutinins in the blood serum of patients, and the skin reactions to the nucleoprotein of the same organisms. We have also demonstrated that there is no correlation between the agglutination reaction and the sedimentation rate of the red blood cells. In the present investigation, we interested ourselves in determining whether there is any relationship between the titre of the antistreptolysin and agglutinins for the streptococcus, and skin reactions to the nucleoprotein of the hemolytic streptococcus or the Dick toxin. Five hundred and thirty-six agglutination tests were done, using the same technique as described in our previous work. Two strains of hemolytic streptococci were used as antigens; one strain (NY5) was the same organism from which the streptolysin was derived, and the other (SD1), a second scarlet fever strain.

In the instances in which agglutinins were present in the blood serum, the antistreptolysin titre varied from 20 to 800 units per cc. The antistreptolysin titre might be low with a comparatively high agglutination and the agglutination reaction was absent or present in low titre in some patients with high titres, so that it was not possible to show any correlation between the two. This is illustrated in Figure 4.
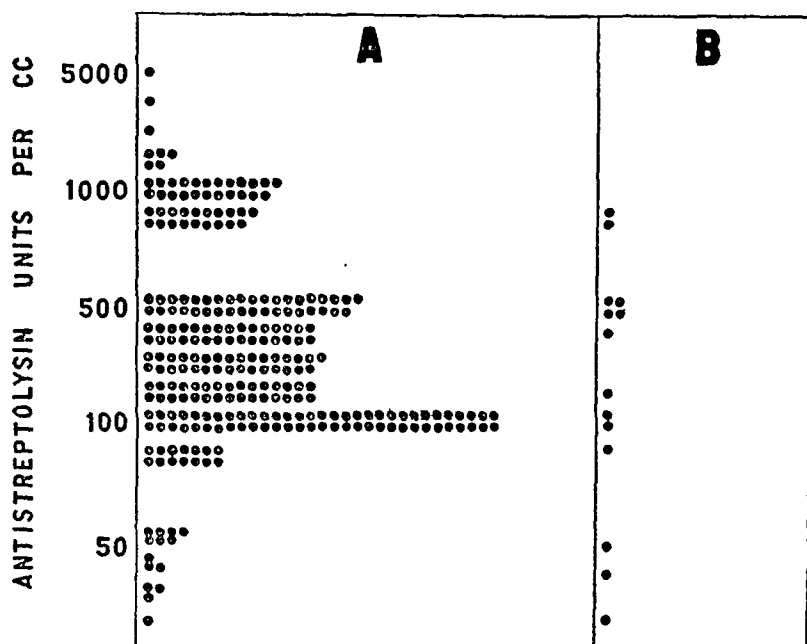

FIG. 4. THE ANTISTREPTOLYSIN TITRE AND STREPTOCOCCAL AGGLUTININS.

Each dot in Column *A* represents the antistreptolysin content of a specimen of serum lacking streptococcal agglutinins. Each dot in Column *B*, the antistreptolysin content of a specimen of serum with agglutinins for the hemolytic streptococcus.

The relationship between the antistreptolysin titre and the skin reaction to the nucleoprotein of the hemolytic streptococcus and to Dick toxin

In fifty-seven patients, the skin reactions to the nucleoprotein of the hemolytic streptococcus (SD_1) was studied as well as the antistreptolysin titre. The patients with negative reactions showed as high an antistreptolysin titre as those with positive skin reactions. The titre in the first group varied from 50 to 1000 units per cc., and in the latter from 20 to 1000 units per cc.

The response to Dick toxin also failed to show any correlation with the antistreptolysin content of the blood serum. The titre of the serum of 53 Dick-negative patients varied between 20 and 1000 units per cc., with an average titre of 360 units per cc. In 4 patients with positive Dick tests the serum contained 100, 100, 200, and 800 units of antistreptolysin per cc. respectively. These findings are in accord with those of Todd,

Laurent and Hill (7) who have shown that the streptolysin and Dick toxin are different substances, just as the antitoxin and antistreptolysin are different. These results are summarized in Figure 5.
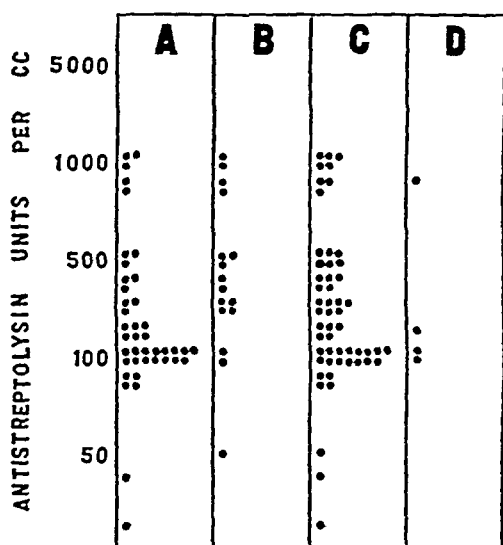

FIG. 5. ANTISTREPTOLYSIN TITRES AND SKIN REACTIVITY TO THE NUCLEO-PROTEIN OF THE HEMOLYTIC STREPTOCOCCI AND TO DICK TOXIN.

Each dot represents the antistreptolysin titre of a serum from a patient who showed: *A*, Negative skin reaction to .01 mgm. of the nucleoprotein of the hemolytic streptococcus; *B*, Positive reaction to .01 mgm. of the nucleoprotein of the hemolytic streptococcus; *C*, Negative skin reaction to 1 S.T.D. of Dick toxin, and, *D*, Positive skin reaction to 1 S.T.D. of Dick toxin.

COMMENT

From the data presented, certain deductions seem justifiable. It is clear that normal individuals have antistreptolysin in their blood serum in varying amounts, and in our experience the titre may remain high for long periods of time without any evidence of active infection with hemolytic streptococci. The variations in the titre of the antistreptolysin in the blood serum of normal individuals and in patients with diseases unassociated with streptococcal infection were greater than those previously recorded in similar groups by Todd (4) (5) and Coburn and Pauli (2). In view of our own previous observation with the reactions of normal individuals to other products of the hemolytic streptococcus, such as the nucleoprotein, this great variation in normals is not surprising. It is important, nevertheless, to remember these differences in assessing the value and significance of the test under consideration.

In patients who have had recent hemolytic streptococcal infections, the antistreptolysin titre is usually higher than for the average normal indi-

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In patients who have had recent hemolytic streptococcal infections, the antistreptolysin titre is usually higher than for the average normal indi-

vidual, and it may increase progressively from week to week. The duration of time that the titre may remain elevated varies in individuals, and this occurs in spite of the fact that all clinical signs of hemolytic streptococcal infections have subsided. As is true with other immune reactions in patients following hemolytic streptococcal infection, there is some irregularity in the formation of antistreptolysin. Thus, we have observed patients who have had active streptococcal infections and in whom the antistreptolysin titre of the blood serum was not elevated during or following the infection.

The titres of the sera from normal individuals and the patients without infection due to hemolytic streptococci were higher on the average than have been reported by other observers (2) (4) (5). The antistreptolysin content of 200 units per cc. in our cases is the average obtained in sera from individuals without infection with the hemolytic streptococcus. Coburn and Pauli (2) accept the presence of this titre to be " a specific indication of infection with the hemolytic streptococcus."

It is not surprising that patients with rheumatic fever show an increase in the antistreptolysin titre of the blood serum as often as patients with proven hemolytic streptococcal infections, since an attack of rheumatic fever is frequently preceded by a hemolytic streptococcus infection. That 21 per cent of the group of patients with rheumatic fever failed to show titres higher than the average for the control groups, would seem to make it justifiable to question the inference that an attack of rheumatic fever is necessarily dependent on a preceding infection by the hemolytic streptococcus.

The titre of the serum of patients with active rheumatoid arthritis was in all respects similar to that for our normal individuals, patients with other types of arthritis and patients with infections not due to streptococci. In these patients, there was no history of a recent streptococcal infection, and this was supported by the low antistreptolysin titre of the blood. It seems evident that the symptoms and signs of active rheumatoid arthritis in the cases we examined were not preceded or associated with an active hemolytic streptococcus infection.

SUMMARY AND CONCLUSIONS

1. In proven hemolytic streptococcal infections the blood serum generally contains antistreptolysin in higher titre than in normal individuals or than in patients with infections caused by other micro-organisms.

2. Patients with acute rheumatic fever, who have had a streptococcal infection, have antistreptolysin titres in their sera comparable to those observed in scarlet fever, erysipelas or acute follicular tonsillitis.

3. Rheumatoid arthritis and certain other forms of joint disease are not accompanied by an increase in the antistreptolysin titre of the blood serum.

3. Antistreptolysin was shown to be an antibody separate and distinct from streptococcal antitoxin or the antibodies responsible for streptococcal agglutination. Antistreptolysin is not related to the skin sensitivity to the nucleoprotein of the *Streptococcus scarlatinae.*

We acknowledge our thanks to Dr. E. H. Place of the South Department of the Boston City Hospital for convalescent scarlet fever serum, and to Dr. A. B. Wadsworth of the New York State Department of Health and Dr. W. G. Malcolm of the Antitoxin Laboratory of the Commonwealth of Massachusetts for horse sera. We also thank Miss Marjorie Jewell, Miss Eleanor Fleming and Miss Jane Locke for technical assistance.

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STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS. V. NORMAL VALUES IN FEMALE SUBJECTS

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Studies of the total pulmonary capacity and its subdivisions have been made in this clinic during the last two years, in an attempt to obtain a quantitative estimation of the degree of functional disability in cases of chronic pulmonary disease. It was found that the normal values gathered from the literature were insufficient to afford an adequate basis for the detection of pathological alterations, and preliminary studies were consequently made in healthy subjects. The results of measurements made in fifty normal male subjects have been presented in previous papers (11), (12). The data show definite limits of variation for the relative values of some of the subdivisions of the total pulmonary capacity, as well as a definite correlation between the pulmonary capacity and combined physical and radiological measurements of the chest. By means of these measurements the pulmonary capacity in a given normal adult male subject can be predicted with a fair degree of accuracy. Since it seemed probable that the same formula can not be applied in the case of normal female subjects, a similar series of observations has been made in them. The results are presented in this communication.

METHODS

As in the previous study, the total pulmonary capacity and its subdivisions were measured by means of Christie's method of oxygen dilution without forced breathing. Complete details will be found in that author's original description (5), and further details as to its use in this clinic are given in the first paper of this series (11). It may be mentioned, that we have become more convinced of the desirability of measuring the *residual air* by this method, rather than the volume of air remaining in the lungs at the end of a normal expiration (called " functional residual air " by Christie and " mid capacity " by us).[2] By

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making duplicate measurements, it was found that better checks were obtained by measuring the residual air, especially since graphic registration of the forced expiration was made at the beginning and at the end of the period of mixing with the contents of the spirometer so that the degree of cooperation of the subject in deflating to the residual level could be accurately ascertained. It has also been observed that a more thorough mixing takes place between the air in the lungs and the oxygen in the spirometer, because of the deep inspiration which follows forced expiration at the beginning and the smaller volume of air contained in the lungs at the residual level. The method as described by Christie may be used advantageously nevertheless, in subjects who are unable

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plicability of this method in the determination of the residual air in such cases as pulmonary emphysema, where this subdivision of the pulmonary capàcity is expected to be increased. In such a case the attainment of complete mixing between the gas mixture in the spirometer and the air in the lungs may be questioned. It has been found in these pathological conditions, as well as in normal people, that repeated duplicate determinations check closely, providing that the time of the rebreathing period (seven minutes) remained constant. Our results then are in agreement with those of most investigators who found that at the end of five to seven minutes there is complete mixing in all cases. Prolongation of this time (to 8, 10 or 12 minutes) leads to an almost constant and proportional increase in the value found for the residual air. It appears that these increasing values are possibly not due to improper mixing but rather to the continuous excretion of nitrogen from the blood into the alveolar air, as shown by Campbell and Hill (J. Physiol., 1931, 71, 309).

The above findings stress the importance of keeping constant the time of the rebreathing period in all determinations. In this series of cases, and in those already reported, the rebreathing period has been from seven to eight minutes.

From the investigations of Hill, Long and Lupton (Proc. Roy. Soc., B, 1924, 97, 84) and Campbell and Hill (J. Physiol., 1931, 71, 309) Christie has estimated that approximately 65 cc. of nitrogen are excreted from the blood into the spirometer-lung system during the seven minutes of rebreathing, and corrects for this factor by subtracting this volume from the final result. The formula given by Christie is as follows:

$$x = \frac{y \, (a - b)}{79.1 - y} - d - 80$$

where $x =$ the lung volume in cc.; $y =$ percentage of nitrogen in the circuit at end of experiment; $a =$ oxygen in the spirometer at beginning of experiment in cc.; $b =$ oxygen absorbed during experiment in cc., and $d =$ dead space in spirometer circuit in cc.

According to the above formula the nitrogen excretion has been corrected only in terms of gas volume added to the system during the rebreathing time, disregarding the fact that it has increased the nitrogen percentage found in the system at the end of the experiment. The formula was developed by equating the volume of nitrogen in the system at the beginning of the experiment against the volume present at the end. By taking into account, in Christie's original equation, any volume of nitrogen (n) excreted into the spirometer-lung system and solving for x the following is obtained:

$$x = \frac{y \, (a - b) - 100n}{79.1 - y} - d.$$

to cooperate satisfactorily in making the maximum effort necessary to deflate the lungs to the residual level.

In most instances two estimations were made. and the results corrected for temperature (37° C.) and complete saturation with water vapor. All observations were carried out in the afternoon after a preliminary rest of at least 20 minutes, the subjects being in the recumbent position with two pillows to serve as a head rest.

A measurement of the respiratory "dead space" was also made in each case under conditions of rest, and in the calculation of this volume the Haldane-Priestley formula:

$$\text{Dead space} = \text{Tidal volume} - \frac{\text{Tidal volume} \times \text{per cent } CO_2 \text{ expired air}}{\text{per cent } CO_2 \text{ alveolar air}} - k$$

(where k is the dead space of the apparatus—in our case 40 cc.) was used. In the collection of the alveolar air sample, a modification of the Fridericia (7) method was employed because of the difficulties usually met with in obtaining a true alveolar air sample from untrained subjects with the well known Haldane-Priestley method. The apparatus used is shown in Figure 1. A mouthpiece. attached to the apparatus by a short and thick rubber tubing. is provided with a valve which allows free and unobstructed passage of the air forwards. but which prevents the return of the air if the subject attempts to inspire before the sample is trapped in the apparatus. The stopcocks have bores 6 mm. in diameter and thus offer very little resistance to the passage of air through them. The whole apparatus is mounted on a wooden frame, and is connected below to a mercury reservoir which is suspended by a hook on the back of the frame. This frame is fitted to slide into a specially constructed table mounted on casters, so that the whole may be wheeled in front of the subject who sits comfortably in a chair. The sample of the alveolar air is obtained in the following manner: The apparatus is set up as shown in Figure 1. Stopcock S^1 connects the rubber tubing of the mouth piece to bulb A, while stopcock S^2 connects this bulb and F with the outside air. The connecting link F of the apparatus is filled with mercury up to stopcock S^2. The subject holds the mouthpiece in his hand and after a few minutes of normal breathing. immediately before a normal expiration is concluded. he is asked quickly to place the mouthpiece in between his lips and make a forced and prolonged expiration. At its conclusion, stopcocks S^1 and S^2 are quickly turned with S_2 connecting F and outer air so that the last part of the expiratory air is trapped in bulb A. The frame is then placed on a support close to the Van Slyke manometric apparatus for the transfer of the gas sample. The mouthpiece and connecting tube are removed. and a glass capillary tube. bent at right angles at both ends, is con-

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Therefore if 65 cc. are excreted during the time of the experiment we believe the formula should be:

$$x = \frac{y(a - b) - 6500}{79.1 - y} - d.$$

The application of the latter formula will give lower values for the residual air than those obtained from the former. Consequently the values given in this series of cases, as well as in the preceding ones, for this subdivision of the pulmonary capacity are probably 100 to 150 cc. too high. Since this is a constant, and probably not a very significant error, it does not invalidate our results, and permits comparative studies to be made with pathological cases.
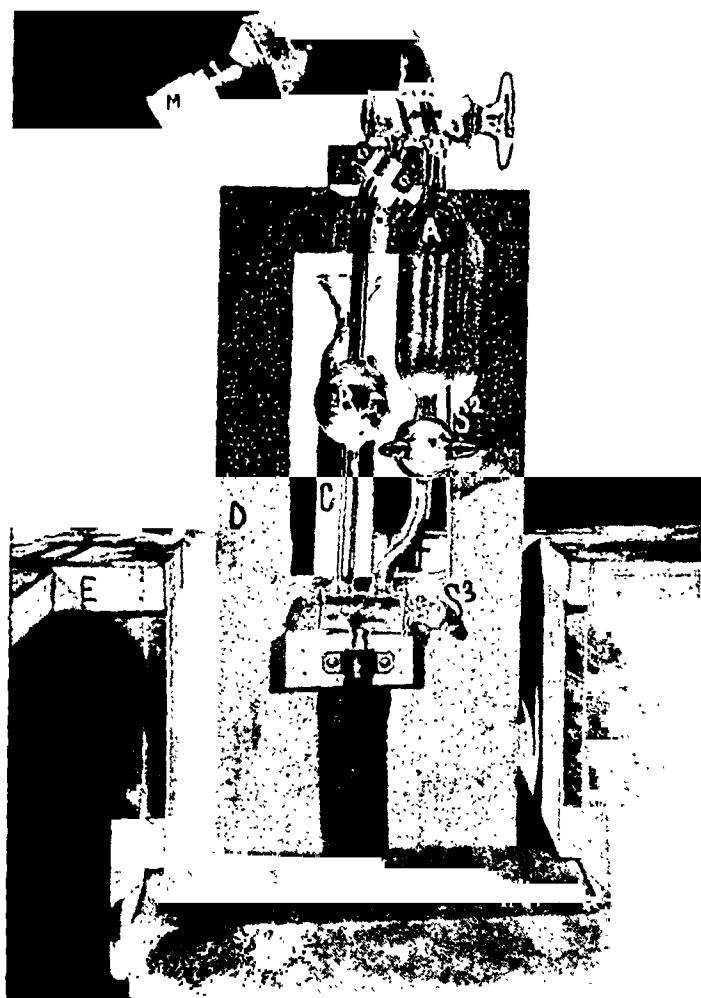
FIG. 1.　APPARATUS USED FOR OBTAINING ALVEOLAR AIR SAMPLES

M. Mouthpiece.　V. Expiratory valve.　A. Bulb of about 60 cc. capacity. B. Bulb of about 25 cc. capacity.　C. Mercury reservoir.　S^1. Stopcock with double bore (one $=$ 6 mm. wide).　S^2. Three way stopcock.　S^3. Stopcock with double bore.　D. Wooden frame.　E. Table.　F. Connecting tube between stopcocks S^2 and S^3.

nected by means of a short thick rubber tubing.　The other end is provided with a rubber tip which fits into the receiving cup of the Van Slyke apparatus. Stopcocks S^1 and S^3 are turned appropriately, and by raising the mercury reservoir the connecting tube and half of the receiving cup are filled with mercury from bulb B.　The air sample is then transferred, either by previously making a vacuum in the 50-cc. chamber of the Van Slyke apparatus and then establishing a communication with the air sample, or by allowing the mercury of the reservoir to displace the samples from bulb A into the chamber.

Incidentally, it may be mentioned that we have found it convenient to use gas sampling apparatus of the type just described (without stopcock S^2).　The double bulb arrangement facilitates not only the washing of any dead space

between the sampler and the gas to be obtained, but also the transfer of the sample to the Van Slyke manometric apparatus for analysis.

Samples were taken in the sitting position because of the larger volume of reserve air in this posture, thus facilitating the process of obtaining true alveolar air. The results showed the mean value of the alveolar CO_2 percentage in this series to be 5.77 ± 0.04 per cent. The tidal air was obtained immediately afterwards with the subject in the same posture. The subject breathed through a mouthpiece from the room air into a Tissot spirometer of 100-liter capacity, which was provided with a recording pen and kymograph. Thus the depth, as well as the rate, of the breathing was recorded. During several minutes of preliminary breathing the dead space of this spirometer was washed with expired air. Tidal air was then collected over a period of from three to four minutes, so that the respiratory dead space as calculated by the formula just given corresponds to the *average tidal volume* during that time. The mean value of the CO_2 percentage of the tidal air was 3.50 ± 0.03 per cent in the fifty cases.

External chest measurements were taken in all subjects, and the "chest volume" calculated from the product of the three dimensions (depth, width and height) according to the method of Lundsgaard and Van Slyke (16). Complete radiological measurements were also made, according to the special technique described in a previous publication (12), which consists of obtaining a double exposure radiograph of the chest, first at maximum expiration and immediately afterwards at maximum inspiration, the subject being in recumbency. The area of the lung fields in square centimeters, multiplied by the corresponding depth (in cm.) of the chest, measured externally, is designated the "radiological chest volume." Theoretically, the latter should represent more accurately the true value of the chest cavity, since it takes into consideration the diaphragmatic level, than any value derived from external measurements alone. Furthermore, from the same film it is possible to estimate the expansion of the lungs from the difference between the areas of the lung fields at maximum expiration and inspiration; the lateral expansion of the chest; the excursion of the diaphragm; and finally, the degree of rib movement.

<div align="center">MATERIAL</div>

Measurements of the total pulmonary capacity and its subdivisions have been made in a series of fifty healthy female subjects, varying in age from 18 to 34 years, with a mean of 23.1 years. The physical characteristics (Table I), show wide variations in the size of the body and the chest, as well as in the shape of the latter. No selective criterion as to any of these characteristics was used in accepting these subjects, so it would seem probable that these cases are fairly representative of the various constitutional types usually encountered at this age period. All of them were submitted to physical and fluoroscopic examination of the chest.

These subjects were students either of the College for Women of the University of Rochester, or of the School of Nursing (Strong Memorial Hospital) of the same university.[3]

TABLE I

Age and physical characteristics of the subjects examined

	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
Age, *years*..............	23.1 ±0.32*	3.4 ±0.23*	14.6	18 – 34
Body height, *cm*..........	163.4 ±0.40	4.2 ±0.28	2.6	152 –177.5
Body weight, *kilo*.........	57.2 ±0.89	9.4 ±0.63	16.4	42 – 83.2
Body surface area, *cm.²*.....	160.0 ±1.29	13.6 ±0.92	8.5	136 –189
Chest volume, *liter*........	6.44±0.11	1.15±0.08	17.9	4.60– 9.41
Chest index $\frac{Depth}{Width} \times 100$..	71.9 ±0.69	7.3 ±0.49	10.1	57.2 –100.0

* Probable error.

Normal values given in the literature [4]

The literature of the subject, with the exception of measurements of the vital capacity, offers relatively few values of the total pulmonary capacity and its subdivisions in normal female subjects. Quite frequently the results obtained in both male and female subjects are presented together. This seems undesirable, for it appears from the results of this investigation that differences between the sexes exist. A study of the values recorded in the literature is further complicated, partly on account of variations in the methods and technique used, and partly because of lack of uniformity in body posture. The nomenclature of the subdivisions of the pulmonary capacity is furthermore by no means standardized.

In Table II are summarized the results of the more important series of determinations made in normal female subjects since the investigation of Lundsgaard and Van Slyke (16) in 1918. There are included seven cases reported by these investigators; eight cases by Lundsgaard and Schierbeck (17) and seven cases by Binger (3), a total of 22 cases. The nomenclature differs from ours in that the "mid capacity" represents the

---

[4] It may be convenient to summarize briefly the nomenclature which we have adopted:

Residual air is the amount of air remaining in the lungs after the fullest possible expiration.

Mid capacity is the amount of air remaining in the lungs after a normal expiration.

Vital capacity is the amount of air expired in the fullest possible expiration following the deepest possible inspiration. Vital capacity is the sum of the complementary and reserve volumes.

Total capacity is the sum of the residual air and the vital capacity.

Complementary air is the volume of air inspired from the position of mid capacity to that of maximum possible inflation. It includes the tidal air.

Reserve air is the amount of air expired from the mid capacity position to maximum possible deflation.

TABLE II

Normal values of pulmonary capacity from the literature *

Pulmonary capacity	Absolute values			
	Mean	Standard deviation	Coefficient of variation	Variations
	liters	*liters*	*per cent*	*liters*
Total capacity..............	4.41	0.62	14.0	3.08–5.50
Vital capacity..............	3.18	0.33	10.3	2.20–3.88
Complementary air..........	1.70	0.32	18.8	1.20–2.31
Reserve air.................	1.52	0.28	18.2	1.00–2.08
Mid capacity†..............	2.76	0.40	14.4	1.88–3.57
Residual air................	1.23	0.28	22.5	0.75–1.75

Relative values (total capacity 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Vital capacity..............	72.0	4.6	6.4	62–82
Complementary air..........	37.7	3.9	10.4	30–46
Reserve air.................	34.0	4.3	12.7	24–46
Mid capacity...............	62.5	3.8	6.2	54–70
Residual air................	28.2	5.5	19.4	18–40

Relative values (vital capacity 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Complementary air..........	52.0	5.2	9.9	40–64
Reserve air.................	48.2	3.9	8.0	36–60
Mid capacity...............	62.5	3.8	6.1	70–108
Residual air................	40.4	9.7	22.5	24–60

* Summary of 22 measurements made in female subjects collected from values reported by Lundsgaard and Van Slyke (16) ; Lundsgaard and Schierbeck (17) ; and Binger (3). Measurements made in sitting position throughout.
† Volume of air in the lungs midway between a normal expiration and inspiration.

volume of air in the lungs midway between a normal expiration and inspiration, and as a consequence the values of this subdivision, as well as those of the complementary air and the reserve air, are not comparable. All the measurements were made, furthermore, in the sitting posture, and for this additional reason they cannot be compared with our results since it has been demonstrated (2), (3), (13), (22) that change in body posture alters chiefly the mid capacity and the complementary and reserve volumes.

RESULTS OF THIS INVESTIGATION

In Table III are summarized the results of the determinations of the total pulmonary capacity and its subdivisions in the fifty healthy adult female subjects. The absolute and the relative (subdivisions expressed
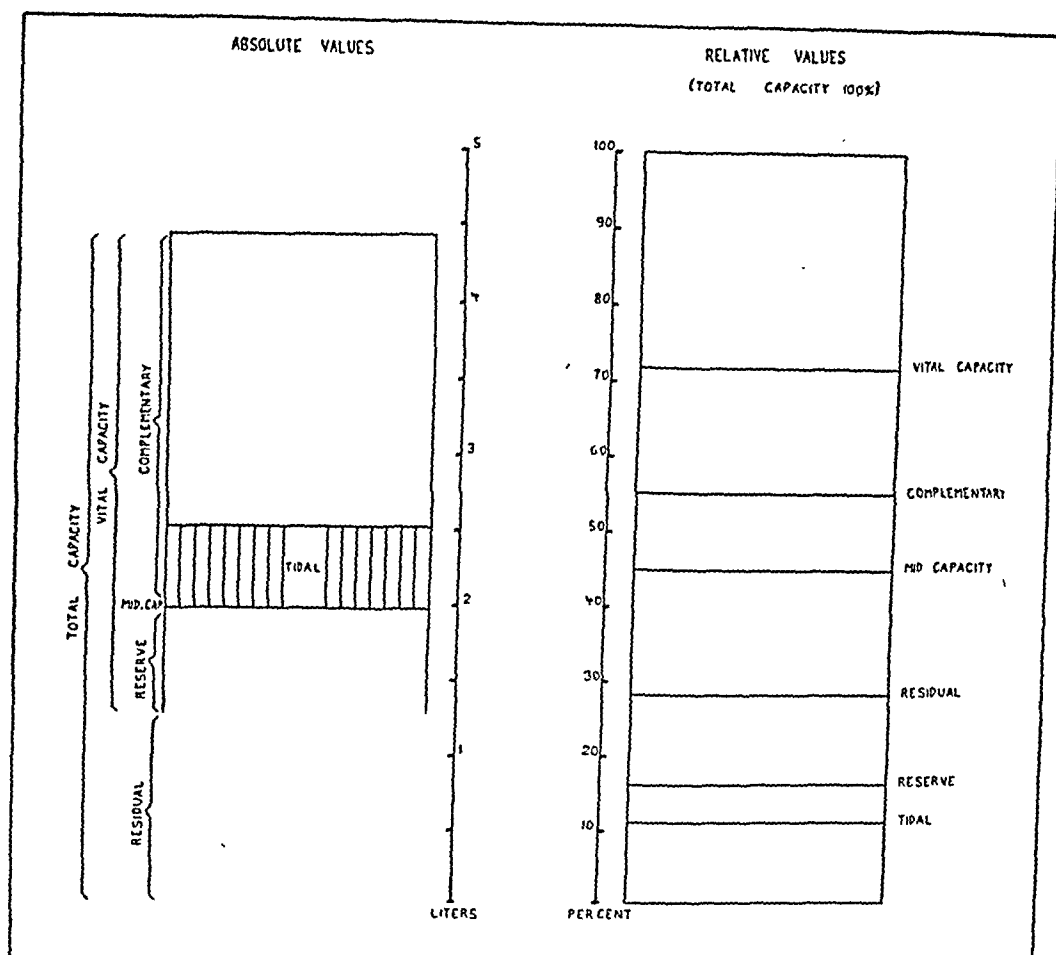
FIG. 2.　MEAN ABSOLUTE AND RELATIVE VALUES OF PULMONARY CAPACITIES
IN 50 NORMAL FEMALE SUBJECTS

as a percentage of the total and vital capacities) mean values, together
with their deviation from the mean and the extreme variations, are pre-
sented.

Absolute values observed. The *total capacity* had a mean value of
4.41 ± 0.06 liters and a standard deviation of 0.59 ± 0.04 liter, indicating,
therefore, a total variation of about 27 per cent. The variations fall
between 3.33 and 6.38 liters. These values agree closely with those col-
lected from the literature, respectively 4.41 and 0.62 liters for mean and
standard deviation, with a total variation of 28 per cent.

The *vital capacity* also varied markedly. Its mean value was 3.14 ±
0.04 liters, with a standard deviation of 0.41 ± 0.03 liter, giving a total
variation around the mean of 26 per cent. This mean value corresponded
almost exactly with that calculated from the literature, although in our
series a somewhat higher degree of variation was found.

The *mid capacity* fluctuated widely. A mean value of 1.98 ± 0.04
liter, with a standard deviation of 0.40 ± 0.03 liter and a total variation
of about 40 per cent were obtained. Its extreme values were 1.29 and

TABLE III

Measurements of pulmonary capacity in 50 healthy female subjects

Pulmonary capacity	Absolute values			
	Mean	Standard deviation	Coefficient of variation	Variations
	liters	*liters*	*per cent*	*liters*
Total capacity.....................	4.41±0.06*	0.59±0.04*	13.3	3.33–6.38
Vital capacity.....................	3.14±0.04	0.41±0.03	13.0	2.28–3.95
Complementary air..............	2.42±0.03	0.36±0.02	14.8	1.70–3.32
Reserve air........................	0.73±0.02	0.19±0.01	25.8	0.28–1.42
Mid capacity......................	1.98±0.04	0.40±0.03	20.1	1.29–3.30
Residual air.......................	1.25±0.03	0.31±0.02	24.6	0.67–2.46

Relative values (total capacity = 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Vital capacity.....................	71.6±0.49	5.2±0.35	7.2	61.5–82.8
Complementary air..............	55.1±0.52	5.4±0.36	9.8	46.1–70.3
Reserve air........................	16.1±0.35	3.7±0.25	22.9	5.9–26.2
Mid capacity......................	44.7±0.50	5.3±0.36	11.8	29.6–53.8
Residual air.......................	28.3±0.48	5.0±0.34	17.6	17.2–38.5

Relative values (vital capacity = 100 per cent)

	per cent	*per cent*	*per cent*	*per cent*
Complementary air..............	77.0±0.72	7.6±0.51	9.8	64.1–92.2
Reserve air........................	22.9±0.43	4.6±0.31	20.1	7.8–35.9
Mid capacity......................	63.3±1.06	11.2±0.76	17.6	38.8–85.5
Residual air.......................	40.2±0.98	10.3±0.69	25.6	19.3–62.7

* Probable error.

3.30 liters. Comparison cannot be made with the values found in the literature on account of the different classification used.

The *residual air* presented even greater variations, ranging between 0.67 and 2.46 liters (however, a volume of more than 2 liters was found in one case only). Its mean value was 1.25 ± 0.03 liter; the standard deviation 0.31 ± 0.02 liter, and consequently the total variation from the mean value was about 50 per cent. Again the mean value and the standard deviation compared closely with the corresponding figures found in the literature (1.23 and 0.28 liter respectively).

The *complementary* and *reserve volumes* had mean values of 2.42 ± 0.03 and 0.73 ± 0.02 liters respectively. They also showed marked fluctuations, especially in the case of the reserve air in which the total variation from the mean exceeded 50 per cent.

It is of interest that the *variations* shown in the absolute values in these female subjects correspond very closely with those found in the group

of male subjects previously reported from this clinic (11). The coefficients of variation are almost similar in both series, although the mean absolute values differ markedly in the two sexes, as must be expected.

Relative values. The values found for the various subdivisions of the lung capacity may conveniently be expressed as percentages of the total capacity. As has been shown above, the absolute values of the total pulmonary capacity and its subdivisions differ widely among individuals; on the other hand, it has been found that, as in male subjects, the values for the various subdivisions relative to the total capacity have normally a fixed range of variation for all individuals. The ratio $\frac{\text{Vital capacity}}{\text{Total capacity}} \times 100$ had a mean value of 71.6 ± 0.49 per cent, with a standard deviation of only 5.2 ± 0.35 per cent, giving a total variation from the mean of less than 15 per cent. It may be recalled that in the series of normal males a similar constancy in the variations of this ratio was observed, indicating, therefore, a rather close relationship between vital capacity and residual volume as component parts of the total capacity. The constancy of this ratio is further confirmed on calculation of the results collected from the literature (Table II). The mean value is 72.0 per cent, with a standard deviation of 4.6, giving a total variation of about 13 per cent. The ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ presented corresponding fluctuations. It varied between 17.2 and 38.5 per cent, with a mean value of 28.3 ± 0.48 per cent, and a standard deviation of 5.0 ± 0.34.

The mid capacity showed wide but well defined variations in its relative value. A mean value of 44.7 ± 0.50 per cent, and a standard deviation of 5.5 ± 0.36 were obtained. The complementary and reserve volumes varied markedly as percentages of the total capacity, especially in the case of the latter.

The relationship between the different pulmonary capacities as expressed by the correlation coefficients [5] may be seen in Table IV. The highest correlation is between the total and the vital capacities. The smaller subdivisions of the pulmonary capacity have also been expressed as percentages of the vital capacity. There are marked variations however, in these ratios (Table III). It is more useful, and in better agreement with most investigators, to use the total capacity as a basis for a comparison of the relative values of its components.

Measurements of the respiratory "Dead space"

The investigation of the respiratory "dead space," measured by the Haldane-Priestley formula (8) from the tidal volume and the CO_2 per-

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[5] Correlation coefficients are significant only when they exceed the probable error multiplied by three, and the correlation is proportionally better as coefficients approach the value of 1.

TABLE IV

Correlation between the different subdivisions

Capacities correlated	Correlation coefficient
Total capacity and vital capacity	$+ 0.8860 \pm 0.0205$*
Total capacity and complementary air	$+ 0.7485 \pm 0.0418$
Total capacity and reserve air	$+ 0.4657 \pm 0.0742$
Total capacity and mid capacity	$+ 0.7916 \pm 0.0351$
Total capacity and residual air	$+ 0.7146 \pm 0.0465$
Vital capacity and complementary air	$+ 0.8742 \pm 0.0222$
Vital capacity and reserve air	$+ 0.4942 \pm 0.0722$
Vital capacity and mid capacity	$+ 0.5129 \pm 0.0701$
Vital capacity and residual air	$+ 0.3518 \pm 0.0829$
Complementary air and mid capacity	$+ 0.2635 \pm 0.0883$
Complementary air and reserve air	$+ 0.0643 \pm 0.0941$
Complementary air and residual air	$+ 0.3647 \pm 0.0823$
Mid capacity and reserve air	$+ 0.5684 \pm 0.0641$
Mid capacity and residual air	$+ 0.8762 \pm 0.0261$
Residual air and reserve air	$+ 0.1994 \pm 0.0944$

* Probable error.

centages of the tidal and alveolar air were made in the sitting posture during rest (Table V). The volume of the "dead space" in our series had a mean value of 144 ± 6.48 cc., corresponding to a mean tidal volume of 0.48 ± 0.02 liter, with a mean respiratory rate and ventilation per minute of 15 ± 0.19 and 6.86 ± 0.15 liters respectively. This observed value for the respiratory "dead space" corresponds very closely to those found by most investigators.

Concerning the true value of the respiratory "dead space," there is a great deal of controversy in the literature. In 1911 Siebeck (21) found that the "dead space" varies considerably in different individuals, and that it is increased during hyperpnea following muscular work. A year later Douglas and Haldane (6) concluded that the respiratory "dead

TABLE V

Measurements of respiratory dead space in 50 healthy female subjects

	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
Dead space, cc.	144 ±6.48*	68±4.59*	47.2	41-449
Tidal volume, liters	0.48±0.02	0.14±0.01	29.1	0.30–0.98
Respirations per minute	15±0.19	2±0.13	13.3	10–21
Ventilation per minute, liters	6.86±0.15	1.54±0.10	22.4	4.24–13.06
O₂ consumption per minute, cc.	242.5 ±3.05	32±2.16	13.2	152–320

* Probable error.

FIG. 3. VITAL CAPACITY, MID CAPACITY AND RESIDUAL AIR EXPRESSED IN PERCENTAGE OF THE TOTAL CAPACITY

The dots indicate observations on 50 individuals grouped about the mean values.

space " is not a fixed anatomical quantity, but rather a physiological variable, closely and proportionally related to the depth of the breathing, being greater during hyperpnea with large tidal volumes, and less during quiet breathing. These findings were later confirmed both by Haldane (9), who believed that the variations are brought about by passive stretching of the atria (beyond the terminal bronchioles), and by Henderson, Chillingworth and Whitney (10), who made use of several different methods, and found that all yielded similar results in showing a close correlation with

the depth of the breathing. They also showed that the respiratory "dead space" undergoes rhythmic variations in the same individual breathing with approximately the same tidal volume. These variations were found to be sometimes as high as 30 per cent.

These conclusions have, however, been criticized by Krogh and Lindhard (14), and also by Pearce and Hoover. (18), who put forward evidence to show that the respiratory "dead space" has a fixed volume in any individual, and is not influenced by the depth of the breathing. Their criticisms were based mainly on the contention that the Haldane-Priestley method for obtaining alveolar air is faulty, especially during hyperpnea. Later these criticisms have to some extent been modified. Krogh (15), although stating that under ordinary conditions of breathing there is very little variation in the respiratory "dead space," found evidence of increments amounting to as much as 100 cc. when very deep breaths were taken. Pearce and Hoover (19) in 1920 presented several observations which appeared to indicate that there is a definite and constant increase in the volume of the "dead space" with very deep breathing, although the increase was not so marked as that found by Haldane. They also concluded that the increment in volume of the "dead space," with increasing tidal volumes, is not a linear function of the depth of the inspiration, but a powered function. Several years ago Aitken and Clark-Kennedy (1) made fractional analysis of a single expiration during muscular activity, measuring the CO_2 percentages of the various samples taken and calculating from them the respiratory "dead space." They also observed a moderate increase as the depth of the breathing increased, although not of the same magnitude as that observed by Douglas and Haldane.

In this series, considerable variations have been found. The standard deviation was 68 ± 4.59 cc. (mean value of 144 ± 6.48 cc.) with a total variation of about 95 per cent. On close analysis the respiratory dead space showed a definite correlation with the tidal volume, particularly when the latter was great (Table VI and Figure 4). With a tidal volume between 0.30 and 0.50 liter, the corresponding dead space varied between 50 and 150 cc., and there was no definite correlation demonstrable between the two. When the tidal volume was above 0.50 liter the volume of the

TABLE VI

Relationship of respiratory dead space to tidal volume

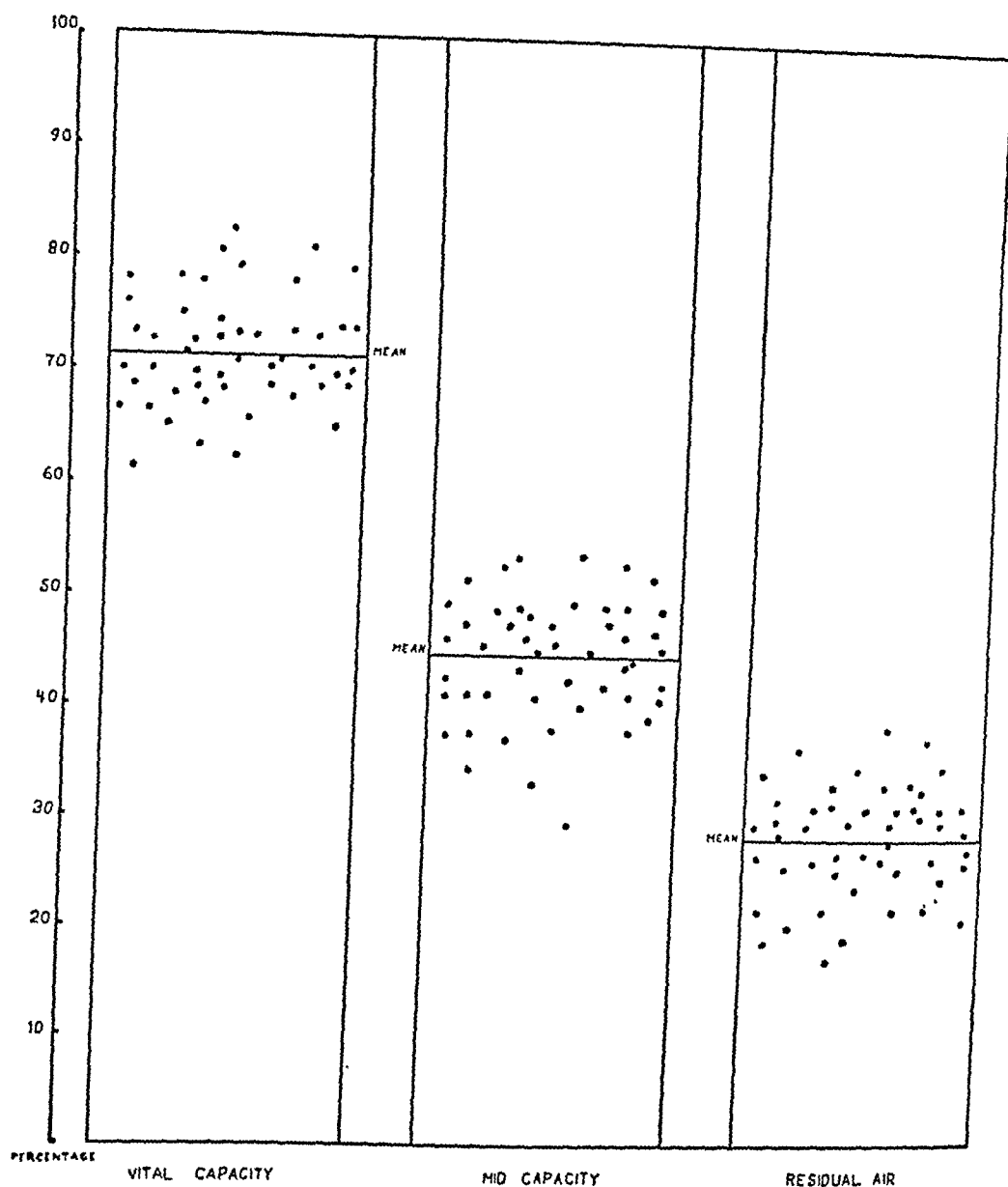| Number of cases	Tidal volume liters	Average dead space cc.
15	0.30–0.39	114
20	0.40–0.49	119
8	0.50–0.59	161
2	0.60–0.69	223
3	0.70–0.79	244
0	0.80–0.89	
2	0.90–0.99	355

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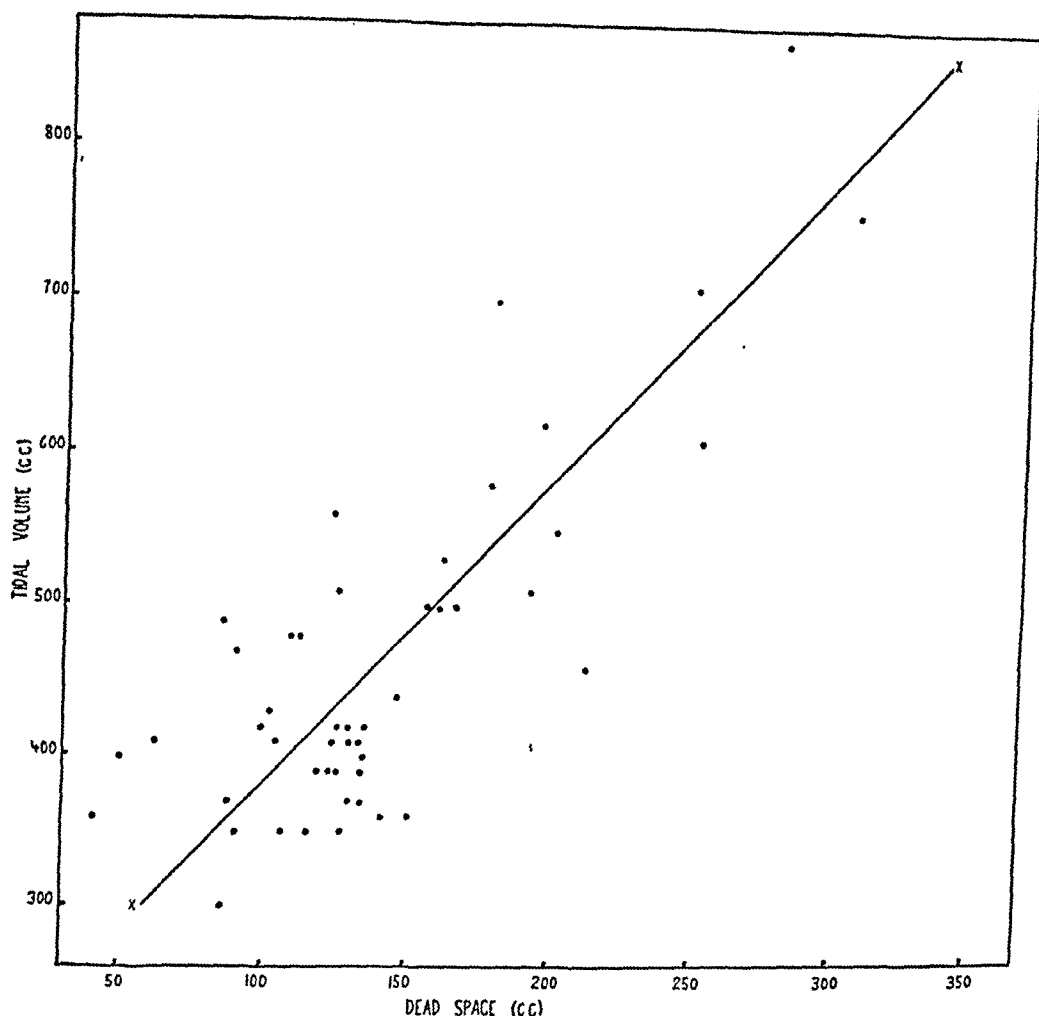| Number of cases	Tidal volume liters	Average dead space cc.
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2	0.60–0.69	223
3	0.70–0.79	244
0	0.80–0.89	
2	0.90–0.99	358

FIG. 4.   CORRELATION BETWEEN THE TIDAL VOLUME AND THE RESPIRATORY
"DEAD SPACE"

The curve x———x represents the regression line derived from the correlation coefficient.

dead space was, however, definitely higher, and continued to increase in proportion to the depth of the breathing.   The correlation coefficient[6] between the tidal volume and the respiratory dead space was $+ 0.8224 \pm 0.0308$.   The correlation was linear. as the correlation ratio was 0.8397, giving an insignificant $\int n^2 - r^2$ of 0.0288 ± 0.0313.

We have also observed, as Haldane has pointed out, that the variations in the volume of the dead space were independent of the respiratory rate. Using the method of partial correlation, it is found that if a constant respiratory rate in all fifty cases is assumed, the standard deviation corresponding to the value of the dead space will be only slightly reduced, becoming 66 cc. instead of 69 cc., i.e. a reduction of only 2.5 per cent.

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[6] The regression equation derived from this correlation coefficient is as follows:

Dead space, cc. = (Tidal volume, liters × 494.9) — 89.9

On the other hand if a fixed tidal volume in all cases is assumed the corresponding standard deviation falls to 39 cc., i.e. a reduction of 43 per cent. The influence of the depth of breathing on the volume of the respiratory dead space is thus made strikingly apparent.

It seemed probable that a relationship exists between the respiratory " dead space " and the mid capacity (amount of air remaining in the lungs after a normal expiration). Observations were made in twelve cases in which the mid capacity was measured in the sitting posture a few minutes after the measurement of the dead space. In these twelve cases the average " dead space" was 149 cc. corresponding to an average tidal volume of 0.50 liter. The correlation coefficient between the two was $+ 0.9023 \pm$ 0.0391. If the respiratory " dead space " is correlated directly with the mid capacity the insignificant correlation coefficient of $+ 0.3387 \pm 0.1881$ is obtained. Applying again the method of partial correlation. and assuming a constant tidal volume in all twelve cases, the correlation coefficient rises, however, to $+ 0.6320 \pm 0.1349$, which indicates a definite relationship between the mid capacity and the volume of the " dead space." In other words, in any two subjects, if both breathe with the same tidal volume, the one with the larger mid capacity will tend to have the larger dead space. Assuming constant tidal and mid capacity volumes in this series of twelve cases, the standard deviation of the value of the respiratory " dead space " falls from 76 cc. to 25.4 cc., i.e. a reduction of 66.6 per cent. This quantity would seem to represent the true variation of the volume of the " dead space," independent of the influence of the tidal volume and mid capacity.

It is of interest finally, briefly to mention the results of measuring the respiratory " dead space " calculated in 41 cases on the basis of the O_2 percentages of the tidal and alveolar airs, according to the formula (10) : Dead

$$\text{space} = \text{Tidal volume} - \left(\text{Tidal volume} \times \frac{20.93 - O_2 \text{ per cent tidal air}}{20.93 - O_2 \text{ per cent alveolar air}} \right).$$

In these 41 cases, the mean oxygen percentages of the tidal and alveolar airs were 16.98 ± 0.05 and 14.14 ± 0.08 per cent respectively. The corresponding mean respiratory " dead space " was 158 ± 10.51 cc., in contrast to the value 147 ± 7.25 obtained by using the CO_2 percentages for the calculation. The finding of a higher value for the volume of the " dead space " on the basis of the oxygen percentages agrees with the observations of Haldane (9). and Henderson. Chillingworth and Whitney (10), who explained the difference as due to the diffusion of CO_2 in considerable amounts from the walls of the mouth. trachea and bronchi.

A closer comparison between the values obtained for the respiratory " dead space " calculated respectively from the CO_2 and the O_2 percentages of the tidal and alveolar airs in our 41 cases may be summarized as follows: In 11 cases (26.8 per cent) the " dead space," as found by both methods, agreed within 10 cc. or less; the " dead space " calculated from the O_2

percentages in 18 cases (43.9 per cent) was 10 cc. or larger; and, in 12 cases (29.2 per cent) was 10 cc. or more smaller. The larger values obtained from using the oxygen percentages were chiefly observed in those cases with large tidal volumes. Thus, in 30 cases in which the tidal volume was below 0.50 liter, the "dead space" calculated from the O_2 percentages was on an average only 0.3 cc. per case larger than that calculated from the CO_2 percentages; but in the remaining 11 cases in which the tidal volume was above 0.50 liter, the "dead space" by the O_2 method was 30.5 cc. larger on the average than when based on the CO_2 percentages. The greatest differences (106 cc. and 123 cc.) were observed in two cases, which also showed the highest tidal volumes (0.76 and 0.91 liter respectively). These findings are consistent with the explanation advanced by Henderson, Chillingworth and Whitney, since it would seem likely that the greater the tidal volume the greater the chance of increased CO_2 diffusion into the respiratory passages.

The variations in the values obtained for the respiratory "dead space" were more marked when measurements were made on the basis of the oxygen percentages. In the 41 cases so investigated, the coefficient of variation was 63.2 per cent, as compared with the value 48.7 per cent obtained when the "dead space" was calculated from the CO_2 concentration in the tidal and alveolar airs.

Correlation with physical and radiological measurements

In the case of the total capacity, the highest correlation observed is that given with the "radiological chest volume" (area of the lung fields at maximum inspiration multiplied by the depth of the chest in the same respiratory position). The correlation coefficient is $+ 0.6294 \pm 0.0573$ (Table VII). The next most significant correlation is with the area of the lung fields at maximum inspiration, where the correlation coefficient is $+ 0.6218 \pm 0.0585$. Correlations with the external measurements of the chest, represented by the chest volume (external), with the body height, weight and surface area are all lower and, in some instances, statistically insignificant.

The vital capacity also shows its highest correlation (and, incidentally, the highest correlation in the total series) with the "radiological chest volume." The correlation coefficient is $+ 0.7073 \pm 0.0476$. Similarly, its next best correlation is that shown with the area of the lung fields, while correlations with the external chest volume and with other physical characteristics are again comparatively low or valueless. The mid capacity and the residual air have no correlation with the external chest measurements at the corresponding respiratory positions of mid capacity and maximum deflation. The observed correlations are in complete agreement with, and confirm those previously found in adult male subjects (12). In that series, the total capacity also was found to be best correlated with the "radiological

TABLE VII

Correlation of pulmonary capacities with physical and radiological measurements

Characteristics correlated	Correlation coefficient
Total capacity and body height	+ 0.5926 ± 0.0613*
Total capacity and body weight	+ 0.1707 ± 0.0924
Total capacity and body surface area	+ 0.2965 ± 0.0863
Total capacity and chest volume (external) (maximum inspiration)	+ 0.3520 ± 0.0829
Total capacity and area of lung fields (maximum inspiration)	+ 0.6218 ± 0.0585
Total capacity and radiological chest volume (maximum inspiration)	+ 0.6294 ± 0.0573
Vital capacity and body height	+ 0.5134 ± 0.0677
Vital capacity and body weight	+ 0.2604 ± 0.0883
Vital capacity and body surface area	+ 0.3378 ± 0.0843
Vital capacity and chest volume (external) (maximum inspiration)	+ 0.3292 ± 0.0850
Vital capacity and area of lung fields (maximum inspiration)	+ 0.5983 ± 0.0612
Vital capacity and radiological chest volume (maximum inspiration)	+ 0.7073 ± 0.0476
Mid capacity and chest volume (external) (mid)	+ 0.1959 ± 0.0917
Residual air and chest volume (external) (maximum expiration)	+ 0.1770 ± 0.0924

* Probable error.

chest volume," the correlation coefficient being + 0.6366 ± 0.0566 as compared with + 0.6294 ± 0.0573 observed in this series. The vital capacity in the male series similarly showed its highest correlation with the "radiological chest volume ": + 0.7174 ± 0.0467, as compared with + 0.7073 ± 0.0476 obtained in this investigation. The other correlation coefficients also agree very closely in the two series, confirming the fact, already emphasized (12), that the pulmonary capacity is most closely correlated and may be predicted with a high degree of accuracy using combined radiological and external chest measurements ("radiological chest volume "). The observation in normal female subjects as in normal male subjects, of poor correlation between the vital capacity and the body surface area is further evidence that this measurement constitutes an unsatisfactory basis for estimating the corresponding normal vital capacity, and this seems particularly the case in individuals in whom there is an imperfect balance between body height and weight.

In female subjects we have again been unable to confirm the findings of Lundsgaard and Van Slyke (16), which seemed to indicate the existence of a close correlation between the total pulmonary capacity and its subdivisions with the chest volume calculated by external measurements of the chest. It is evident that such a method fails to take into account the level of the diaphragm, and consequently gives an imperfect representation of the true size of the chest cavity.

Prediction of the normal capacity of the lungs

Since of all the characteristics investigated both in male (12) and female subjects the highest correlation discovered has proved to be that existing between the vital capacity and the "radiological chest volume," the method of predicting the total pulmonary capacity and its main subdivisions in any given case remains substantially the same. It must be emphasized, however, that since the absolute as well as the relative values differ in the sexes, the formulae also differ. Knowing only the "radiological chest volume" the corresponding vital capacity may be predicted by means of the regression formula[7] calculated from the correlation coefficient between these two quantities, while in turn other subdivisions and the total capacity may be derived from the predicted vital capacity by making use of the fixed relative values found for these components.

The method of calculation may be illustrated by the following example:

(a) The area of the lung fields at maximum inspiration, as measured on the radiological film, is 520 square centimeters. The external anteroposterior diameter of the chest in the same respiratory position is 20 cm. Therefore the "radiological chest volume" is $520 \times 20 = 10.40$ liters.

(b) The corresponding vital capacity would then be (according to the regression formula): $(10.40 \times 0.214) + 0.95 = 3.18$ liters.

(c) The total capacity would be (taking into consideration that the vital capacity is 71.6 per cent of the total capacity): $\frac{3.18}{71.6} \times 100 = 4.44$ liters.

(d) The residual air is equal to the total capacity minus the vital capacity: $4.44 - 3.18 = 1.26$ liters.

(e) The mid capacity would be (taking into account the fact that the mid capacity is 44.7 per cent of the total capacity): $\frac{4.44 \times 44.7}{100} = 1.98$ liters.

Between the predicted and the observed values, there is, as in the male series, a very close correspondence (Figure 5). A variation of more than 15 per cent from the calculated values of the observed total and vital capacities, and of more than 30 and 40 per cent in the mid capacity and residual volumes respectively may be considered abnormal. The limits of variation for the latter volumes may appear to be considerable, but the volumes are relatively small and show wide physiological variations in the absolute values.

Since the investigation of normal male subjects was reported a large number of observations in cases of chronic pulmonary disease has been

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[7] The regression formula is:

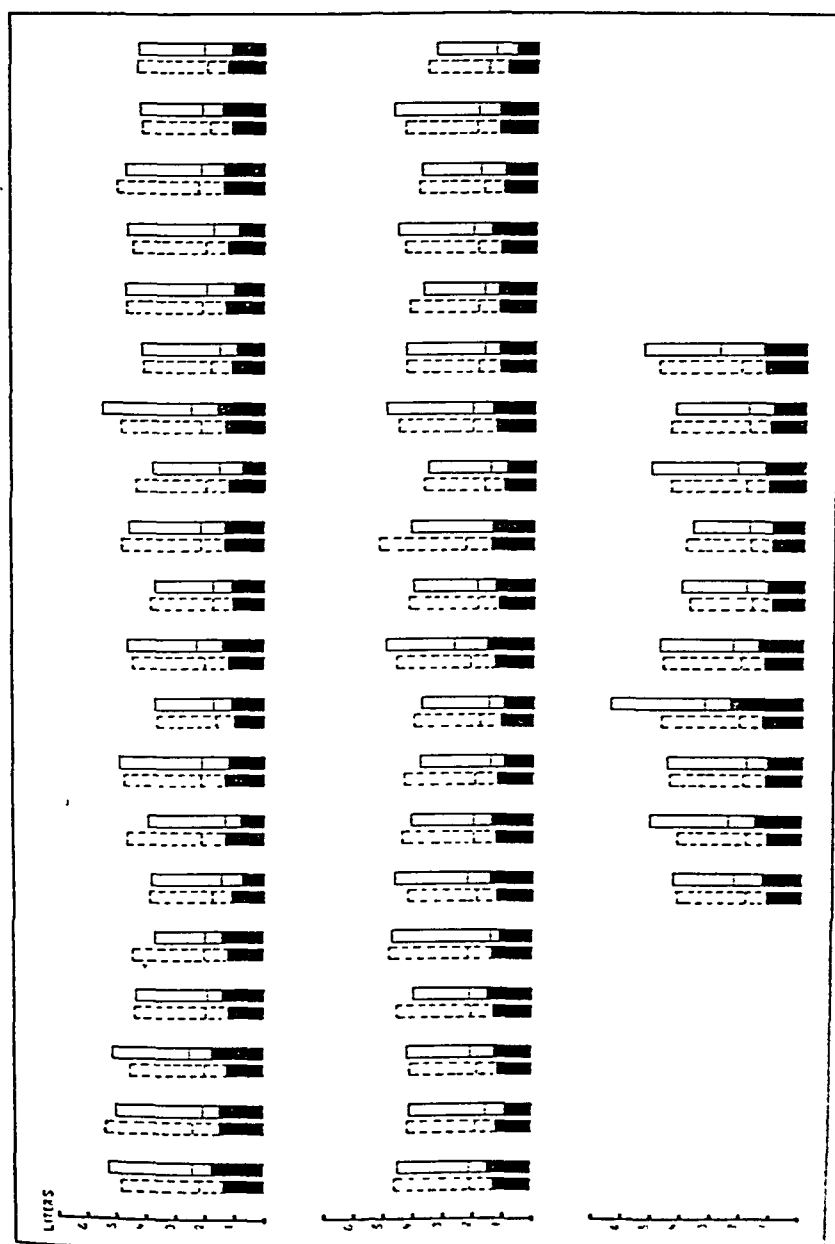Vital capacity, liters = ("radiological chest volume" liters $\times$ 0.214) + 0.95

FIG. 5. CALCULATED AND OBSERVED PULMONARY CAPACITY IN 50 HEALTHY FEMALE SUBJECTS

Each case is represented by two columns: On the left, with broken lines, the calculated value is given; on the right is represented the observed value.   The line dividing the vital capacity is at the level of mid capacity.

The black area represents the residual air, while the white space above is the vital capacity.

accumulated.   The application of the limits of normal variations has been found to be useful in the detection of the pulmonary abnormality, and in the appreciation of the degree of functional respiratory disability.

Chest expansion and pulmonary capacity

The expansion of the chest estimated by external measurements showed marked variations, and bore no relationship to the absolute or relative pulmonary capacities.   Radiological measurements made with a doubly exposed film furnished, on the other hand, more definite criteria for the appreciation of the normal range of expansion.   In the ratio $\frac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$, the mean value was 63.6 ± 0.49 per cent, the standard deviation only 5.1 ± 0.34, and the resultant total variation only 16 per cent.   These findings are similar to those observed in normal male subjects in whom the mean value of this ratio was 62.2 ± 0.42, the standard deviation 4.4 ± 0.30, and the total variation 14 per cent, and afford confirmation, therefore, of the restricted and fixed limits of variation of this ratio in normal subjects.   According to these observations, if this ratio exceeds 75 per cent in a female subject a reduction in the expansion of the chest may be assumed to exist.   This ratio of course fails to take into consideration the expansion in the anteroposterior diameter of the chest, but since this is very nearly equal to the lateral expansion (2.9 and 3.1 cm. respectively as measured externally) it may be inferred that alterations in the former may possibly be reflected equally in the latter and thus appreciated in the radiological measurement.

It is of interest to find in female, as in normal male subjects, that the ratio $\frac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$ bears a relationship to the corresponding relative pulmonary capacity.   If it is correlated with $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ the correlation coefficient is $+0.5189 \pm 0.0695$, indicating that diminished chest expansion tends to be accompanied by a high proportion of residual air to the total capacity, or in other words by probably defective alveolar ventilation.

Other radiological measurements of chest expansion presented marked variations, but it seems probable that on occasion they offer additional information in explanation of the mechanism of decrease in expansion.   The excursion of the diaphragm, the expansion in the width of the chest and the rib movement may, for example, be considered to be abnormally reduced if they are less than 2.5, 2.0 cm. and 12° respectively.

Individuals with broad chests and high diaphragmatic levels (hypersthenic type) frequently have small volumes of reserve air.   The same observation was made in male subjects.   This type of chest tends to exhibit a small pulmonary area in roentgenograms and correspondingly

lower pulmonary capacity, when compared with chests of the long slender type in which the diaphragmatic level is low (asthenic type).

TABLE VIII

Measurements of chest expansion

	External measurements			
	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
Lateral expansion, *cm*............	3.1±0.07	0.8±0.05*	25.8	1.5–5.0
Anteroposterior expansion, *cm*	2.9±0.07	0.8±0.05	27.5	1.5–4.5

Radiological measurements

Excursion of diaphragm, right, *cm*..	5.0±0.12	1.3±0.09	26.0	2.4–7.4
left, *cm*...	5.0±0.12	1.3±0.09	26.0	1.5–7.3
Expansion in chest width, *cm*.....	3.4±0.06	0.7±0.04	20.6	1.2–5.5
Rib movement, *degrees*.........	24±0.40	4.2±0.28	17.4	11–33
$\dfrac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$	63.6±0.49	5.1±0.34	8.0	51.7–75.6

* Probable error.

SUMMARY AND CONCLUSIONS

Measurements of total pulmonary capacity and its subdivisions have been made in 50 healthy female subjects of an average age of 23 years. The physical characteristics of the subjects examined are fully presented. The technique and methods described in previous papers (11), (12) have been used. Normal values gathered from the literature are summarized.

The results obtained in healthy female subjects, confirm the findings and conclusions reached in a similar study of fifty healthy male subjects, but at the same time indicate the necessity of utilizing separate normal values for the two sexes.

Measurements of the respiratory "dead space" have also been made, using the Haldane-Priestley formula in calculation, involving the CO_2 and the O_2 percentages of the tidal and alveolar air, and the tidal volume.

The observations presented lead to the following conclusions:

1. In normal adult female subjects there are marked individual variations in the absolute values of the total pulmonary capacity and its subdivisions. They are closely correlated, however, with the "radiological chest volume," calculated from radiological and external chest measurements. A formula has been developed which permits the prediction of the normal pulmonary capacity and its main subdivisions in any given case.

2. A variation from the predicted values of more than 15 per cent in the observed total and vital capacities, and of more than 30 and 40 per cent in the mid capacity and residual air respectively, may be considered to be significant and beyond the normal limits of variation.

3. The vital capacity, mid capacity and residual air fluctuate within definite limits if expressed in percentage of the total capacity. Alveolar ventilation is probably defective in any case in which the ratio $\dfrac{\text{Vital capacity}}{\text{Total capacity}} \times 100$ is lower than 60 per cent, or if correspondingly the ratio $\dfrac{\text{Residual air}}{\text{Total capacity}} \times 100$ is higher than 40 per cent.

4. The volume of the respiratory "dead space" is independent of the rate of the breathing, but varies with its depth. An increase in "dead space" becomes evident whenever the tidal volumes exceed 0.50 liter. The correlation is linear and positive. The "dead space" is also found to be correlated with the mid capacity, although to a lower degree.

5. The respiratory "dead space" calculated on the basis of the oxygen content of the tidal and alveolar air is larger than that calculated from the corresponding carbon dioxide content, when the tidal volume exceeds 0.50 liter.

6. The chest expansion may best be appreciated from measurements of the areas of the lung fields at maximum expiration and inspiration shown on a radiological film. If the ratio $\dfrac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100$ is higher than 75 per cent the chest expansion may be considered to be abnormally small. There is a positive correlation between this ratio and the proportion of residual air to total capacity, indicating that a decrease in the expansion of the chest tends to be accompanied by a less effective pulmonary ventilation.

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THE DIGESTION OF BEEF PROTEINS IN THE HUMAN STOMACH [1]

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It is at least 2500 years since man first exhibited curiosity about the mechanism of the process by which he digests his food. Since that time the stomach has been regarded as playing, if not the most important, at least a very important part in the process. It is a curious fact that, while as a result of considerable research since the early seventeenth century, we know a great deal about the part played by that portion of the digestive tract described as the intestines, we know little or nothing of the part performed by the stomach itself. It is true, of course, that an immense amount of information has been gained in the last quarter of a century about gastric secretions and gastric motility. Extensive investigations have also been made into the mechanism and the nature of the products of peptic digestion *in vitro*, but little or no knowledge has been gained of the extent and nature of gastric digestion of protein in the living stomach. Still less is known of disturbances which may occur in the gastric digestive process in disease, although in at least one disease it seems possible that disturbance of this process may play an important rôle. The most striking precursor and concomitant of pernicious anaemia is the condition described as achlorhydria. It has been known that achlorhydria is accompanied in many cases by achylia and it is a corollary therefore that in pernicious anaemia gastric digestion is incomplete or entirely absent. There have been no systematic studies of gastric digestion of protein hitherto made in this disease.

In view of the results obtained by Castle and others (1) in the treatment of pernicious anaemia with normal gastric digests and extracts of gastric and other tissues, which suggest that faulty gastric secretion and digestion may be at least a factor in the causation of the disease, it was deemed desirable to carry out an investigation of the extent and character of gastric protein hydrolysis in normal individuals and in a number of abnormal conditions, but especially in simple achlorhydrics and in patients with pernicious anaemia.

A number of preliminary experiments *in vitro* were essential to establish the experimental conditions. These are described below under the

following headings: (1) the rate of hydrolysis; (2) the error introduced by the momentary acceleration of hydrolysis due to increased temperature when, in determining the amount of hydrolysis of beef protein during a given time, boiling was employed to destroy the enzyme; (3) the extent of peptic hydrolysis of beef protein at the average $C_H +$ of the ingested tissue (pH $= 5.5$); (4) the inhibitory effect of bile on peptic digestion.

The extent of hydrolysis was determined by fractional analyses of the digests, according to the method of Wasteneys and Borsook (2). In this method, protein is precipitated by a 2 per cent trichloracetic acid, proteoses by saturated sodium sulphate at 33° C., peptones by tannic acid, and poly-peptides by alcohol. Alcohol precipitation was, however, carried out in only a few cases and in none of these was any measurable amount of amino-acid nitrogen found.

TABLE I

Rate of peptic hydrolysis of beef muscle protein
Suspension approximately 11 per cent

Experi-ment	Enzyme	Time	Hydroly-sis	Protein fractions			
				Protein N	Proteose N	Peptone N	Subpep-tone N
		seconds	*per cent*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
A	1 per cent boiled pepsin	0		61	2	25	12
B	1 per cent active pepsin	30	14.9	52	13	14	21
C	1 per cent active pepsin	130	21.4	48	13	17	22
D	1 per cent active pepsin	180	26.8	45	18	14	23

PRELIMINARY IN VITRO EXPERIMENTS

1. *The rate of peptic hydrolysis of meat*

As a substrate for the experiment, 100 grams of lean beef were ground with sand in a mortar and 800 cc. of water added. This suspension was brought to a pH of 1.35 and to a temperature of 37.5° C. Sample A was removed, boiled pepsin [2] was added to a concentration of 1 per cent, and a fractional analysis made.

To the remainder, pepsin was added to a concentration of 1 per cent. Samples B, C and D were then removed 30, 130 and 180 seconds after the addition of pepsin. Immediately on removal the pepsin was destroyed by adding 1 cc. of concentrated sodium hydroxide.

The results of fractional analyses of these samples are given in Table I.

The concentration of active pepsin used in this experiment is relatively low. It is less than that which is often found in human gastric contents. Even in this low concentration pepsin acts *in vitro* with remarkable rapidity, 27 per cent of the protein having undergone hydrolysis in 180 seconds.

[2] Merck's commercial pepsin.

The rate of peptic hydrolysis is much more rapid with meat than with egg albumen, yet the relation between the different hydrolytic products, as hydrolysis proceeds, is similar.

2. The acceleration of hydrolysis due to temperature when digests are heated to destroy the enzyme

In view of the results obtained in the previous experiment, the possibility suggested itself that in the usual method for destroying enzyme action by rapid boiling, a transitory acceleration of hydrolysis might occur even though the time interval necessary to attain the temperature at which pepsin is destroyed be short.

In order to ascertain whether this acceleration is significant, a mixture of ground lean beef and water was brought to a pH of 1.2. This was divided into two portions. To one portion a solution of boiled pepsin in a concentration of 1 per cent was added. The mixture was boiled and submitted to fractional analysis. To the other sample, unboiled pepsin in the same concentration was added and the mixture was almost immediately boiled and analyzed. The results are given in Table II.

TABLE II

The degree of protein hydrolysis which occurs in a mixture of pepsin and macerated beef muscle during the time taken to raise the temperature of the mixture from room temperature to the boiling point

Fraction	Boiled pepsin and substrate raised to boiling point	Pepsin (active) added to substrate and raised to boiling point
	per cent	*per cent*
Protein N.	56	16
Proteose N.	10	36
Peptone N.	27	27
Subpeptone N.	7	21

Seventy-one per cent of the protein was hydrolysed. This experiment shows clearly that heating the digest in the presence of pepsin near the optimum pH for peptic hydrolysis causes quite marked hydrolysis, even though the time taken to raise the mixture from room temperature to that at which pepsin is destroyed, which is about 72° C. (3), is less than a minute. One must conclude, therefore, that under the conditions of this experiment boiling is an unsatisfactory method of inactivating the enzyme. It should be pointed out, however, that different conditions obtain when a sample is removed from the stomach an hour or more after the ingestion of 100 grams of lean beef and the material is boiled before making a fractional analysis. The enzyme and substrate have here been together for some time at a temperature of 37.5° C., and considerable hydrolysis has already taken place except in those cases where the pepsin content of the gastric juice is low. The speeding up of hydrolysis in the process of bringing the mixture to a boil would, in either condition, be minimized.

This is illustrated by the following experiment. The gastric contents of a patient were removed by stomach tube, one and one-half hours after ingestion of 100 grams of lean beef. The pH of the gastric contents was 1.65. They contained three times as much pepsin as was contained in the artificial digests. The gastric sample was divided into two parts. In one-half the pepsin was destroyed by boiling. In the other half it was destroyed by the addition of alkali. The fractional analyses are given in Table III.

TABLE III

A comparison of the nitrogen fractions in gastric contents after inactivation of the pepsin by boiling and by the addition of alkali

Fractions	Pepsin inactivated by boiling	Pepsin inactivated by alkali
	per cent	*per cent*
Protein N....................	23	25
Proteose N....................	41	40
Peptone N....................	20	22
Subpeptone N..................	16	13

If the sample in which the pepsin is inactivated by alkali be considered as the control, there is a negligible effect produced by meat. In most samples obtained from patients, even less than this amount of hydrolysis by heat would occur because in many cases the gastric contents contain less pepsin and the $C_H +$ is farther from the optimum for hydrolysis. One must conclude, therefore, that although boiling the gastric contents is not the best method for inactivating the pepsin, yet it may be used for gastric contents as the increased hydrolysis of the substrate during inactivation is negligible. In the majority of cases, there was only a limited supply of material obtained from the stomach, and as it was necessary to make several determinations on the same sample, the pepsin was inactivated by boiling.

3. *The peptic hydrolysis of beef protein at pH 5.5*

A question arises in the consideration of achlorhydria. Is there any digestion of meat at the pH of the gastric contents, usually 5.5 to 6, in this condition? In order to test this point a suspension of finely ground meat, adjusted to pH 5.5, was divided into two parts; to one was added boiled pepsin to a final concentration of 1 per cent. No hydrolysis was observed in 3¾ hours. To the other half, active pepsin was added in similar concentration. At the end of 3¾ hours 27 per cent of the protein had been digested. In spite of the possibility indicated by this experiment, it was found, as will be shown later, that very little digestion of protein actually takes place in the stomachs of achlorhydrics.

4. *The inhibitory effect of bile on peptic digestion*

Conflicting opinions are to be found in the literature as to the inhibitory action of bile on gastric digestion, and some experiments were carried out

in an endeavour to decide this question. The rate of peptic hydrolysis of 4 per cent egg albumen in the presence and absence of human bile from the gallbladder was determined, and in order to exaggerate any inhibitory effect of bile the amount of pepsin used in the experiment was relatively small while the amount of bile was considerably greater than that which is usually present in gastric contents as a result of regurgitation from the duodenum. The extent of hydrolysis during a period of four hours was measured. The inhibition of hydrolysis due to bile was, however, too slight to be significant.

HUMAN GASTRIC DIGESTION

In previous investigations of gastric digestion of beef muscle two general methods have been used: the formol titration of the gastric contents according to the method of Henriques and Sörenson (4), or some modification of that method; and the Van Slyke method of amino-nitrogen determination. The first method was used by Zunz (5), London (6), Christiansen (7) and Rehfuss (8). In general these observers found little apparent digestion in the stomach.

The relatively slight digestion found by these observers is probably accounted for by the fact that in peptic digests the ratio of free carboxyl or free amino-nitrogen to total nitrogen, even at equilibrium, is so small and the consequent error is so great, that little or no information can be gained as to the extent of changes which have occurred.

Apart from these investigations there have been few or no attempts at measurement of actual gastric digestion, and in studies of the relative digestibility of foods, workers have usually contented themselves with measurements of the emptying time of the stomach. In the present study of gastric digestion of beef protein the amount of digestion was followed by determining, at a given time after ingestion, the extent to which the protein had been hydrolyzed in the stomach into the various fractions.

It should be pointed out, however, that the amount of protein and nonprotein nitrogen in the gastric contents cannot be assumed to give an absolute measure of the extent to which beef muscle protein has been digested because of the unknown quantity of protein and nonprotein nitrogen contained in the saliva and other secretions of the gastro-intestinal tract, oral to the pylorus. They do, however, give the most accurate picture obtainable of actual gastric digestion of protein.

In this investigation, proven cases of pernicious anaemia and hospital patients suffering from other than gastro-intestinal diseases were used. In selecting the subjects, except in patients with pernicious anaemia, care was taken to avoid using any cases giving a definite history of previous gastro-intestinal disease or acute febrile states, debilitating disease, or any condition in which one might expect temporary achlorhydria. For the purpose of this study, achlorhydric individuals under observation were

divided into two groups: patients with achlorhydria with pernicious anaemia; and patients with achlorhydria without pernicious anaemia.

The subjects were prepared as follows: An ordinary meal was given at 4:45 p.m. on the preceding day, and on the day of the test no breakfast was given. At 9:00 a.m. the subject was given 100 grams of finely ground lean beef adequately flavoured with salt and pepper. The meat was fried to a light brown surface colour and 300 cc. of water was taken with the meat. Ingestion of the meat occupied from ten to fifteen minutes. The period of digestion was taken as the time from the beginning of ingestion to the time at which the removed gastric contents were heated to destroy the enzyme.

In order to obtain the stomach contents a large stomach tube was used. It was occasionally necessary to inject a small amount of water in order to clear the eye of the tube, and an attempt was always made to remove the entire content of the stomach at one time. This was immediately taken to the laboratory and a small amount put aside for pepsin and bile estimations. The remainder was boiled to destroy the pepsin. The boiled material was used for fractional analysis and also for the pH determination. It had already been shown that boiling did not appreciably affect the pH of the gastric contents.

Pepsin was estimated according to the method of Michaelis and Rothstein (9), by comparing the clearing of a suspension of a sulphosalicylic-acid-precipitated serum by a standard pepsin solution with the clearing effected by equal amounts of gastric contents in varying dilution. Human serum was obtained from the excess of serum remaining from samples of blood on which routine Wassermann tests were performed. It was necessary, in the estimations of pepsin, to use a filtrate of gastric contents because the turbidity produced by particles of meat interferes with the reading of the tubes. The dilutions were recorded as units of pepsin.

The standard pepsin solution was made from commercial scale pepsin which, when tested, complied with the U. S. Pharmacopoeia requirements. This solution contained a high percentage of glycerol. It was kept in the refrigerator and retained its activity for long periods of time.

The van den Bergh reaction was found to be sensitive for the detection of bile in the gastric contents. The unfiltered gastric contents were used for the test as it was found that less colour was produced in the filtrates than in the unfiltered material. This is believed to be due to adsorption of the bile on the particles of meat. After the addition of the diazo reagent and alcohol to the gastric contents the suspended particles were filtered off and the colour was estimated in the filtrate. The concentration of the sulphanilic acid and sodium nitrite was increased four times over that used in the standard van den Bergh test, as this was found to increase the sensitivity of the test. These bile estimations were only roughly quanti-

tative but one part of gallbladder bile in two thousand parts of a thick solution of finely ground beef gave a definitely positive test.

Assuming, according to clinical usage, that free hydrochloric acid is absent in cases where the pH is above 4 (that is, alkaline to dimethyl-amino-azobenzol (Toepfer's reagent)), then 90.5 per cent of the normal cases had free acid present in the gastric contents. Four per cent of the normals had gastric contents with pH values from 4 to 4.5, and an average titratable acidity of 17.2 cc. of N/10 acid per 100 cc. Sixty-five per cent of the cases had gastric contents with pH values ranging from 1.5 to 2.5, that is, in the range of pH for optimal peptic activity; for 11 per cent the pH values were within a range in which pepsin is relatively inactive. A few of these cases are not included in Table IV, which shows the correlation between the ex-

TABLE IV

The correlation between extent of hydrolysis of beef muscle protein and pH in human gastric contents

Range of pH	Number of cases	Per cent of total cases	Average pH	Average titratable acidity	Per cent cases showing bile	Average pepsin	Averages			
							Protein N	Proteose N	Peptone N	Subpeptone N
				cc. N/10		*units*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
1–1.5	13	9	1.4	84.0	27	4.0	24	36	19	21
1.5–2	27	18	1.7	67.7	23	3.4	24	32	18	24
2–2.5	38	26	2.2	71.3	20	2.5	25	37	18	19
2.5–3	24	16	2.7	48.9	24	2.1	30	38	19	15
3–3.5	25	17	3.2	40.4	32	0.6	39	35	14	12
3.5–4	6	4	3.7	24.8	40	0.1	61	22	8	8
4–4.5	6	4	4.3	17.2	20	0.0	67	15	9	7
4.5–5	2	1	4.6	7.0	0	0.0	57	11	13	21
5–5.5	3	2	5.2	1.7	0	0.0	73	10	15	3
5.5–6	1	1	5.7		0	0.0	74	9	17	
6–6.5	0									
6.5–7	1	1	6.6		100	0.0	62	13	2	22
7–7.5	0									
7.5–8	1	1	7.7		100	0.0	71	17	6	6

tent of hydrolysis and pH in patients without history of gastric disease or pernicious anaemia. The 11 per cent of achlorhydric individuals found compares closely with the percentage observed by Bennett and Ryle (10). The titratable acidity varies with the true acidity from an average of 84 cc. of N/10 acid per 100 cc. of gastric contents at a pH of 1 to 1.7 cc. at a pH of 5. Methyl red was chosen as the indicator for the titration because its turning point is close to the normal pH of the meat fed to the patients. The large amount of acid secreted in response to the meat stimulus as compared with the stimulus of an Ewald test meal is noteworthy.

Of 129 "normal" subjects tested for bile, 25 per cent showed bile or

traces of bile in the gastric contents. Of the 32 cases showing bile, 14 cases had only a trace. Castle (11) states that bile appeared only occasionally in the gastric contents of normal students to whom meat was fed. Judged by these results and those of Castle, duodenal regurgitation can neutralize gastric contents in only a small percentage of humans. Boldyreff (12), working with dogs, states that the neutralization of gastric contents is normally effected by duodenal regurgitation, and Bolton and Goodhart (13) and Medes and Wright (14) also state that duodenal regurgitation is a normal means of neutralization of acid gastric contents in human subjects, but MacLean and Griffiths (15) seldom found CO_2 or trypsin in the gastric contents of humans, and Shay, Katz and Schloss (16) find that the neutralization of gastric contents by duodenal regurgitation, even when it occurs, is probably insignificant.

The amount of active pepsin in the gastric contents was found to diminish as the acidity decreased, and the highest pH at which active pepsin could be demonstrated was 3.5. This is indicated in Table IV where the cases are grouped according to the pH of the gastric contents within a range comprising 0.5 of a pH. The results are averages of the individual pH groups, and pernicious anaemia cases are excluded.

As would be expected, the amount of protein remaining undigested shows a marked increase at hydrogen ion concentrations less than a pH of 3.5, and the proteose, peptone and subpeptone correspondingly diminish as the acidity decreases. It is not only the decreased acidity, however, which is responsible for the diminution in digestion, but also the absence of pepsin. Of the 147 cases exclusive of pernicious anaemia, 86 per cent showed appreciable amounts of pepsin present, with a considerable digestion of protein. Approximately one-fourth of the cases showed presence of bile.

There is considerable variation in all the factors in those cases in a given pH group. This is illustrated in Table V, which includes cases comprising the first group (pH values 1 to 1.5) of Table IV.

In Table VI an attempt is made to compare the amount of digestion with its duration. There is only a somewhat doubtful correlation shown between the length of time the beef muscle has been in the stomach and the actual digestion. Obviously the factors of hydrogen ion concentration and pepsin content are of greater significance than duration of time in determining the amount of digestion effected. When, however, the effect of time is examined at definite pH values, a less doubtful correlation is shown—Table VII. These results are arranged in groups according to the pH of the gastric contents and within these groups the duration of digestion and amount of digestion are compared. At a given pH there is a general tendency towards more digestion the longer the meat remains in the stomach.

TABLE V

Correlation between digestion and acidity in human gastric contents in the pH range 1–1.5

Case	Duration of digestion	pH	Titratable acidity	Bile	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
	hours		*cc. N/10*		*units*	*per cent of total N*	*per cent of total N*	*per cent of total N*	*per cent of total N*
L	1.45'	1.23	100	++	8	28	26	24	22
I	2.0'	1.26				19	43	27	11
W	1.30'	1.30	87	0	1	23	43	19	15
R	2.15'	1.31	78	0	4	14	46	21	19
M	1.45'	1.34	102	0	2	23	46	13	18
K	1.50'	1.35	79	tr.	3	29	45	1	25
J	1.30'	1.37	94	0	3	30	31	20	19
W	2.20'	1.37	73	0	6	15	29	30	26
H	1.45'	1.38	84	tr.	6	28	48	5	19
F	1.55'	1.41	56	0	3	19	24	(57)	
B	1.55'	1.45	85	0	4	23	37	24	16
P	1.0'	1.46	52	0	4	(59)		12	20
C	2.0'	1.46	119			32	17	25	26

TABLE VI

Comparison of the amount of human gastric digestion with its duration

Protein digested	Average digestion	Average time	Longest time	Shortest time	Number of cases
per cent	*per cent*	*minutes*	*minutes*	*minutes*	
90–100	92	103	140	90	6
80–89	83	103	150	75	28
70–79	73	104	150	75	48
60–69	65	100	135	60	19
50–59	54	93	120	60	13
40–49	44	90	115	70	10
30–39	34	93	120	75	12
20–29	24	89	140	70	11
10–19	14	94	120	50	11
0–9	6	96	135	75	3

Correlation between the age of patients and amount of digestion, pH and titratable acidity was next studied. Table VIII gives the average digestion in the age groups.

In the second, third and fourth decades there is a gradual decrease in digestion, titratable acidity and free acidity as shown by increasing pH. In the fifth, sixth and seventh decades, however, there is more digestion and lower pH than in the fourth decade of life, whereas during the eighth no acidity, a high pH and low digestion prevails. The last two decades include, however, only an insignificant number of cases. Bloomfield and Keefer (17), in studying the secretion of the stomach, found no free acid in three out of five cases from 60 to 70 years of age. In grouping the

TABLE VII

Comparison of digestion in cases having same duration of digestion and pH

Number of cases	Time	Titratable acidity	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
	hours	cc. N/10	units	per cent of total N	per cent of total N	per cent of total N	per cent of total N
pH Group 1–1.5							
4	2.00–2.15	93.5	5	20	33	26	21
6	1.45–2.00	84.4	4	25	38	14	20
2	1.30–1.45	90.5	2	26	37	20	17
1	1.00–1.15	52.3	4	(59)		12	29
pH Group 1.5–2							
2	2.15–2.30	50.2	5	9	16	(75)	
3	2.00–2.15	58.2	3	20	35	(45)	
7	1.45–2.00	77.0	4	28	34	17	22
9	1.30–1.45	68.3	4	31	29	30	19
3	1.15–1.30	64.0	3	23	39	15	23
pH Group 2–2.5							
3	2.15–2.30	25.0	(2)	25	39	10	26
11	2.00–2.15	70.3	2	26	38	18	18
2	1.45–2.00			20	44	(36)	
pH Group 2.5–3							
14	1.30–1.45	74.4	3	21	39	20	21
6	1.15–1.30	81.0	2	23	36	21	19
2	1.00–1.15	84.7	2	24	42	15	9
pH Group 3–3.5							
2	2.15–2.30	28.6	trace	26	30	(44)	
5	2.00–2.15	40.6	1	34	48	4	16
2	1.45–2.00	18.3	trace	45	22	24	9
5	1.30–1.45	46.8	1	42	27	18	12
9	1.15–1.30	45.8	trace	39	27	12	11
1	1.00–1.15	15.9	trace	45	20	27	8
1	.45–1.00		1	31	60	(13)	

patients, they used the period of twenty years and found in their average a gradual decrease in the acidity of the gastric contents. If the above figures were calculated on the same basis, the average titratable acid between 20 and 40 years of age, and 40 and 60 years, would be about the same. Apparently in older subjects the acidity falls rapidly. Dedichen (18) working with over 100 subjects between the ages of 87 and 92 showed that

TABLE VIII

Correlation of age with gastric digestion, pH, and titratable acidity

Age group in years	Gastric contents			Number of cases
	Average pH	Average titratable acidity per 100 cc.	Average * protein digested	
		cc. N/10	*per cent*	
10–19	2.3	68	73	7
20–29	2.5	64	70	33
30–39	3.0	49	60	33
40–49	2.5	56	69	32
50–59	2.7	48	63	13
60–69	2.6	45	68	3
70–79	5.7	0	45	2

four-fifths of the men and three-fifths of the women had *achylia gastrica*.

Grouping the results on patients according to the clinical diagnosis on admission yielded very little information of value, but a few points of interest may be mentioned. Patients with disseminated sclerosis showed good digestion, normal acidity and normal amount of pepsin. Eleven osteoarthritic patients had, on the average, a normal activity of the stomach. This is contrary to the experience of Bell (19). He states that 37.5 per cent of the cases with osteoarthritis showed achylia gastrica; and 12.5 per cent showed low acid content. Two alcoholics showed less acidity and digestion than normal. Psychasthenics had normal secretion of acid and digestion of meat.

The extent of gastric digestion and the bile and pepsin in the gastric contents in achlorhydric individuals without pernicious anaemia[a] and in those suffering from pernicious anaemia is shown in Table IX. The average pH of the gastric contents was 6.7 with a range of from 5.75 to 7.53, and no titratable acid and no pepsin was found. The cases with pernicious anaemia showed less acidity than the achlorhydric cases and no combined acid, while several of the achlorhydrics showed small amounts

TABLE IX

Comparison of gastric activity in achlorhydrics with and without pernicious anaemia

Type	Number of cases	Average pH	Cases with bile	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
					per cent of total N	*per cent of total N*	*per cent of total N*	*per cent of total N*
P.A.	23	6.7	4	0	75	7	6	11
Achlorhydric	13	4.9	2	0	67	12	10	8

[a] Subjects who have not suffered from acute illness recently so as to render them achlorhydric temporarily.

of combined acid. The absence of free acid and the relative absence of titratable acid occurring in pernicious anaemia has been noted by many observers. Bile was present in four out of twenty-three cases. Medes and Wright (14) found that seven out of nine cases of pernicious anaemia showed bile in the gastric contents.

Very little actual hydrolysis of meat protein appears to take place in the stomach of cases with pernicious anaemia. If the amount (approximately 10 to 14 per cent of sub-protein nitrogen) which is usually present in beef muscle be added to the average of 75 per cent of protein nitrogen, it appears that not much more than 10 per cent of the meat protein was hydrolyzed in the experimental period.

The achlorhydric cases without pernicious anaemia showed slightly more digestion. Patients with pernicious anaemia and achlorhydrics without pernicious anaemia, as is well known, discharge ingested foods through the pylorus with much greater rapidity than other individuals. The fact that achlorhydrics digest very little protein in the stomach suggests that measurements of " emptying time " may lead to entirely erroneous conclusions as to digestibility.

If there is anything in the theory that cases of pernicious anaemia are recruited from achlorhydric cases it is conceivable that there is some connection between the continued deprivation of peptic digestion and the disease. A peculiarity common to the achlorhydric patients and those with pernicious anaemia is the relatively high proportion of the subproteose fraction in the gastric contents. This suggests the possibility that such gastric digestion as does occur in these individuals is tryptic and not peptic in character, and is possibly in these subjects due to regurgitation of duodenal contents into the stomach.

In view of the great variation in the amount of digestion and in other factors shown by the different groups, an attempt was made to determine whether single individuals examined more than once under approximately the same conditions would show any constancy in behavior. In Table X are given the results obtained with six individuals each examined on two or more occasions, one day or usually more apart. While the hydrogen ion concentration and total acidity, and to a lesser extent the actual digestion, appear to be somewhat characteristic for the individuals, there is, nevertheless, considerable variation. E. J. M., for example, who was examined on five separate occasions, always several days apart, had total acidities varying from 41 to 90 cc. N/10 per 100 cc. And while the amount of active pepsin in the gastric contents was on four occasions relatively high, on one occasion, when the amount of digestion was correspondingly small, none could be detected. This subject experienced no symptoms of gastric disturbance (other than that caused by withdrawal of the sample of gastric contents) or ill health during the period of the experiments.

TABLE X

Gastric digestion in single individuals on different days

Case	Duration of digestion	pH	Titratable acidity	Bile	Pepsin	Protein N	Proteose N	Peptone N	Subpeptone N
	hours		cc. N/10		units	per cent of total N	per cent of total N	per cent of total N	per cent of total N
A	1.00′	3.0	63	0	2	15	44	33	8
	1.30′	3.6	47	0	tr.	52	27	18	2
	1.45′	2.4				20	44	14	22
B	1.15′	2.4	151	0	4	24	50	6	20
	1.45′	2.3	102	0	2	23	46	13	18
C	2.00′	1.7				29			
	2.00′	2.1				28	52	9	11
D	1.15′	3.4	36	0	tr.	59	27	3	11
	1.15′	2.4		0	1	20	21	40	19
E	1.20′	3.2		0	tr.	78	5	10	7
	1.15′	5.3		0	0	93	1	3	3
EJM	1.45′	2.5	74	++	3	30	29	27	14
	1.55′	2.7	41	+++	2	17	48	20	15
	1.55′	2.4	90	++	4	28	38	7	27
	1.15′	3.3	70	+++	0	67	19	7	7
	1.20′	2.7	51	+	2	24	45	14	17

The examinations of gastric contents made of a relatively large number of cases in the course of this study show, in general, a surprising uniformity in the extent of gastric digestion. It averages, one estimates, about 50 per cent. Even in cases of pernicious anaemia and in achlorhydrics, gastric contents which show no demonstrable pepsin and very low hydrogen ion concentration present from 10 to 20 per cent digestion. It seems that even from normal stomachs 40 to 50 per cent of ingested protein is on the average discharged through the pylorus in an undigested condition, but it is probable that the sojourn of the remaining 60 to 50 per cent in the bath of acid gastric juice may facilitate its digestion in the small intestine. Since under normal conditions at least 99 per cent of ingested protein is absorbed from the gastro-intestinal tract, the failure to complete peptic digestion in the stomach may have little significance so long as the digestive secretions of the small intestines are normal. In this connection, observations made on depancreatized dogs are of interest (20). In these dogs on the average only 60 per cent of ingested protein is absorbed from the gastro-intestinal tract, the remainder being eliminated unhydrolyzed in the faeces. If we may assume that with these animals, as with humans, roughly 50 per cent of the protein leaves the stomach unhydrolyzed, then the failure

to utilize more than 60 per cent of ingested protein in dogs may be due to the fact that in them only protein which has undergone peptic digestion is readily hydrolyzed to an absorbable form while ingested protein, which escapes this digestion, does not undergo further digestion in the intestine of the depancreatized animal. In such animals, therefore, gastric digestion is probably fundamental to proper nutrition.

SUMMARY AND CONCLUSIONS

1. The digestion of protein in the human stomach has been studied in a series of cases, and quantitative fractional analyses of gastric digests are reported.

2. Considerable peptic hydrolysis of meat can occur in the stomach in a relatively short time.

3. There is a very wide variation in the extent of hydrolysis of beef muscle protein in the normal individual. This variation occurs not only in different normal subjects, but also in the same individual.

4. Subjects with pernicious anaemia accomplish very little or no gastric digestion of meat.

5. No pepsin was demonstrated in the gastric contents of achlorhydric cases without pernicious anaemia nor of patients suffering from pernicious anaemia.

6. Achlorhydric cases without pernicious anaemia, however, showed a small amount of gastric digestion. A somewhat smaller amount was found in pernicious anaemia cases.

7. Under the conditions of these experiments, pepsin secretion and acid secretion appear to parallel each other in amount.

8. The pH of the gastric contents in individuals with apparently normal gastro-intestinal tracts, ranges from 1.23 to 6.63. No patients with pernicious anaemia had a pH below 5.75 for their gastric contents.

9. The titratable acidity ranged from 151 cc. of N/10 acid per 100 cc. of gastric contents to zero. As was true of the digestion of protein, it varied considerably in individual cases. In a few subjects on whom more than one determination was made variation was also found.

10. Bile was present in the gastric contents in measurable amounts in about 25 per cent of the normal subjects tested, and in 18 per cent of the subjects with pernicious anaemia.

11. It should be pointed out that these conclusions are based on observations of gastric digestion of meals consisting of meat only, and may be different for similar observations on a mixed dietary.

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CUTANEOUS REACTIONS IN THE DIAGNOSIS OF UNDULANT FEVER

By JACOB D. GOLDSTEIN

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In the majority of instances the diagnosis of undulant fever can be established by the agglutination reaction of the patient's serum. This test, because of its simplicity, the rapidity of its performance, and the high degree of reliability, is at present the procedure of choice as a routine measure. In contrast to this test the cultivation of the infecting organism from the blood or the stool offers far less certainty of success, even in the most experienced hands. Moreover, it is a time consuming procedure.

However, certain important limitations of the agglutination test have been encountered. Carpenter and Boak (1) and Giordano and Sensenich (2) have reported a number of cases, proved bacteriologically to be undulant fever, in which the titres of the anti sera were less than 1 : 30. The former workers also found no demonstrable agglutinins in five of their cases. In Europe a larger number of similar instances have been reported. These uncertainties of the agglutination test, although present in a relatively small number of cases, have stimulated further investigation of the endermic reaction as a diagnostic test in undulant fever.

The studies of Fleischner and Meyer (3) demonstrated that infection of guinea pigs with *B. abortus* always produced cutaneous hypersensitiveness. However, in a series of 75 infants, who were fed upon milk with a high content of *B. abortus,* they did not find any hypersensitiveness of the skin. In 1922, using a broth filtrate (melitene or abortine), Burnet (4) first applied the endermic reaction as a diagnostic test in Malta fever. He considered the result of this test to be conclusive in the diagnosis of the infection in guinea pigs and in man. Simpson and Fraizer (5) in 1929 tested the cutaneous reactions of 10 proven cases of undulant fever and 25 controls with a suspension of *B. abortus* killed by heat. The proven cases all gave positive reactions whereas the controls were all negative. Giordano (6) using a similar antigen found that 25 proven cases exhibited severe local reactions while 99 of 100 control cases were negative. Levin (7) in a series of 365 patients tested, reported that 27 showed positive reactions. Of those who reacted positively 15 were proven by other tests to have brucella infections and 8 had probably had such infections in the past. In

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TABLE I

Positive reactions to whole brucella organisms killed by heat

Sex	Age	Agglutinin titre before test	Fever as result of test *	Complaint of illness after test †	Clinical diagnosis	Regional glandular enlargement	Local necrosis	Occupation	Birthplace	History pertinent to possible exposure
M	*years* 51	1 : 80	0	++	Sacroiliac arthritis with fever	+	+	Meat-cutter	Saratoga Springs, N. Y.	Handled fresh meat for years Drank raw milk
M	37	1 : 10	+++	+++	Duodenal ulcer	++	++	Painter	New Jersey	None
F	22	0	Already febrile	++	Ulcerative colitis	++	+	Office	Rochester, N. Y.	None
F	21	0	++	+	Ureteral stone	+		Waitress Kitchen worker		
F	60	0	+	++	Diabetes	+	−	Home Farm	Italy	Drank cows' raw milk None
F	55	0	0	0	Fracture	++	++	Home Farmer—milk	Italy New York	Drank raw milk Milked cows
M	47	0	++	++	Syphilis	++	++	Home	Mount Morris, N. Y.	Drank raw milk Lived on farm
F	32	0	0	0	Pregnancy	+	+	Shoe factory	Lithuania	Handled cows
F	40	0	+	+	Perineal repair	−	−	Home	Rochester, N. Y.	None
F	32	1 : 80	++	+++	Infected abortion	++	++	Home	Rochester, N. Y.	None
F	38	1 : 20	0	++++	Hysterectomy for myoma			Factory	Rochester, N. Y.	? Raw milk

TABLE I (continued)

Sex	Age	Agglutinin before test titre	Fever as result of test *	Complaint of Illness after test †	Clinical diagnosis	Regional glandular enlargement	Local necrosis	Occupation	Birthplace	History pertinent to possible exposure
	years									
F	57	1:20	0	0	Gynecological patient	–	+	Home	New York	None
M	45	0	+	0	Hernia	+	+	Laborer	Italy	None
M	68	1:160	++	+	Prostatectomy	–	++	Minister	Michigan	Drank raw milk
M	50	0	++	+++	Heart failure	++	+++	Carpenter	N. Y. State	None
F	27	1:40	++	++++	Cystitis	++	+++	Bacteriologist	N. Y. State	Exposed to cultures in laboratory. Drank raw milk
F	26	1:160	++	++	Abortion	++	–	Home	Rochester, N. Y.	None
F	43	0	0	0	Cervicitis	++	–	Home	Rochester, N. Y.	None
F	22	Not done	0	+	Pregnancy	++	–	Home	Lyons, N. Y.	Drank raw milk
M	27	0	++	++++	Diabetes	++	+	Student	Ohio	Cows' raw milk
M	27	0	++	+++	No disease	++	++	Student	Victor, N. Y.	Raw milk
M	25	0	++	+++	Arthritis spine	++	–	Student	Rochester, N. Y.	Raw milk
M	33	0	0	+	No disease	++	+	Student	Schenectady, N. Y.	None
M	26	0	++	++	No disease	++	–	Student	Ohio	None
F	48	1:160	Already febrile		Hypertensive heart disease	+	+	Farm help	Rochester, N. Y.	Cows' raw milk. Good history of undulant fever
F	60	0	++	++	Hypertension	+	+	Home Farm	Sweden	Not clear

* *Fever*
+ 37°–38° C.
++ 38°–39° C.
+++ 39° +.

† *Illness*
+ general malaise
++ malaise and aches
+++ malaise, aches and anorexia
++++ felt like severe "grippe." Required going to bed.

TABLE III

Agglutinin titres after injection of 1/20 cc. of whole organisms killed by heat (½ billion per cc.)

Cutaneous reaction	Titre before test	Subsequent titres *			Cutaneous reaction	Titre before test	Subsequent titres *		
		1	2	3			1	2	3
−	0	7–0			−	20	23–640		
+	80	7–80	11–80	29–160	−	−	23–1280	60–320	142–80
−	−	7–0	26–320		−	−	23–1280	60–320	142–80
−	−	7–0	28–1280		+	−	23–5120	60–2560	142–1280
−	−	7–0	17–0		+	−	23–640	60–640	142–320
−	−	7–0			+	20	23–1280	60–1280	142–80
−	−	7–0	29–160		−	−	23–640	60–320	142–320
−	−	7–0	26–320		−	−	23–320	60–320	142–80
+	−	23–1280			−	−	23–640	60–160	142–80
−	−	7–0			−	−	23–5120	60–2560	142–1280
−	−	27–0			+	20	23–10240	60–1280	142–640
+	−	17–40	27–80		−	−	23–10240		142–160
+	−	5–0	22–640		−	−	23–1280	60–80	142–40
−	−	17–160			−	20			142–160
+	−	10–1280			−	−	23–2560		142–40
−	−	25–640			−	−	23–10240	60–640	
−	−	28–80			−	−	23–320		142–160
−	−	30–0			−	40	23–5120		142–320
+	80	19–2560			−	−	23–320	60–80	142–20
−	−	17–0			+	−	23–1280	60–1280	142–320
−	−	11–160			−	−	23–10240	60–320	142–80
−	−	11–1280			−	−	23–5120	60–160	142–80
+	−	12–320			−	20	23–5120	60–160	142–80
−	−	12–1280			−	−	23–5120	60–1280	142–640
+	80	23–640			−	−	23–320	60–160	
−	40	14–80	37–320		−	−	8–160		
−	−	14–320			−	−	22–640		
−	20	11–320			−	−	7–0		
−	20	14–0	31–2560		−	−	11–0		
−	−	16–160			−	−	8–320		
−	−	14–1280							
−	−	23–1280	60–320	142–160					
+	−	23–640	60–320	142–40					
−	−	23–2560	60–320	142–160					
+	−	35–1280							
+	40	23–320	60–1280	142–160					
+	−	23–5120		142–80					
+	−	23–1280	60–640	142–160					
−	−			142–80					
+	40	23–5120	60–320	142–320					

* 26–320 means 26th day, titre 1 : 320.

out having any evidence of disease which can be recognized clinically as undulant fever. If the reactivity of the skin produced in man as a result of a brucella infection is comparable to that produced similarly in guinea pigs, then the test probably has a specific diagnostic value. From the analysis of all the data of our patients who gave positive reactions, *it seems*

TABLE IV

Agglutinin titres after intradermal injection of 1/20 cc. fat-free antigen containing 1/250 mgm. bacterial protein per test dose

Cutaneous reaction	Titre before test	Subsequent titres	
—	—	28–0	
—	—	28–20	
—	—	28–0	
—	640	*28–160	50–80
—	80	*28–160	50–160
—	—	28–0	
—	40	28–0	
—	20	28–20	
—	—	28–0	
+	—	28–0	
—	—	28–80	
—	20	28–0	
—	—	28–20	
—	—	28–0	
—	—	28–0	
—	—	28–20	
+	—	28–0	
—	—	28–0	
—	—	28–0	
—	20	28–0	
—	40	28–40	50–10
—	20	28–40	
—	—	28–0	
—	—	28–20	
—	—	28–20	50–0
—	—	28–20	
—	—	28–20	
—	40	28–40	
—	—	30–20	
—	—	30–20	50–0
+	—	14–0	
+	20	10–80	

* Students previously tested with whole organisms. Cutaneous reaction remained negative following both tests.

advantageous to use the endermic reaction as a diagnostic measure not only in cases of unexplained fever without anti-abortus agglutinins, but also in obscure cases with histories of past febrile illnesses of unknown etiology, vague gastro-intestinal disorders and unusual types of arthritis.

Because it induces less severe local and general reactions, the use of the fat-free material is preferable to that of the whole organisms. The fat-free antigen has an additional advantage in that it has a lower capacity to produce agglutinins, and in consequence the subsequent appearance of agglutinins is less likely to give rise to confusion. Sera of very high agglutinin content can be produced in non-hypersensitive individuals by one

or two intradermal injections of a suspension of whole organisms. This fact may be made use of, by employing such an individual as a donor, in the hope of conferring some passive immunity to a patient.

SUMMARY

1. The endermic reactions of 253 patients were tested by the intradermal injection of whole *B. abortus* organisms killed by heat. Twenty-six individuals or 10.3 per cent of this group reacted positively.

2. The reactions of 92 patients were similarly tested with *B. abortus* organisms which had been extracted with alcohol and ether. Nine or 9.6 per cent of this group gave positive reactions to the " fat-free " antigen.

3. The " fat-free " antigen produced fewer general reactions and less severe local reactions than did the " heat-killed " vaccine. It did not materially increase the anti-abortus agglutinin titre of the serum.

4. The endermic reaction is recommended for use as a supplementary procedure for the diagnosis of undulant fever, not only in those patients who have an unexplained fever, but also in those afebrile individuals without anti-abortus agglutinins in their serum who may be suspected of having symptoms referable to a brucella infection.

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THE DISTRIBUTION OF BLOOD PHOSPHORUS AFTER SUP-PRESSION OF RENAL FUNCTION [1]

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The studies reported here are part of a series dealing with the distribution of phosphorus in the blood in a number of pathologic conditions which are known to affect the phosphorus metabolism of the body. In earlier papers attention has been directed especially to the organic acid-soluble fraction of the blood phosphorus, designated " ester P," and to changes of its concentration in relation to other chemical constituents of the blood. Following experimental high intestinal obstruction in dogs there were found marked increases of ester P in the blood cells, as well as of inorganic P in the plasma (1, 2). In another study, it was found that following the administration of large doses of irradiated ergosterol to rabbits the increases of inorganic P in the plasma—changes already made familiar through the work of previous investigators—were accompanied by increases of ester P in the cells which were of greater magnitude than the increases of inorganic P (3). While the mechanism of such increases of ester P in the cells appeared obscure, it was recognized that in these conditions the partial suppression of renal function might be a factor influencing the changes observed. In further study of this phase of the problem, several experimental procedures known to bring about acute suppression of renal function in animals have been employed. In this paper are described some of these experiments, dealing with effects of: 1.—Bilateral ligation of the ureters in rabbits and dogs; 2.—Mercuric chloride poisoning in dogs; 3.—Diphtheria intoxication in rabbits and dogs.

METHODS

The methods of blood examination and chemical analysis used have been described in two previous papers (1, 3), with the exception of total base and pH determinations. Total base was determined by the method of Stadie and Ross (4), as modified by Kirjan (5). For the pH measurements we are indebted to Dr. H. W. Robinson. The pH of the serum

was determined, as drawn, by the Cullen colorimetric method (6), and this measurement was controlled by equilibrating each sample of serum at a known CO_2 tension in order to obtain the " c " correction for that serum (7).

Two samples of diphtheria toxin (designated respectively Number 390 and Number 38M[51]) were obtained from the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Health through the courtesy of Dr. Benjamin White, Director. The minimum lethal dose of both toxins was 0.015 cc., and the effects of both were practically the same except that one, Number 390, produced more paralysis in the late stages of intoxication than did the other.

Full-grown large rabbits (2.0 to 4.0 kgm.) were used in order to minimize the effects of repeated bleeding. They were of both sexes and several breeds, and were fed a commercially prepared rabbit food with small amounts of green vegetables. The dogs were full-grown mongrels, of both sexes, and were fed meat scraps, bones and prepared dog biscuit.

The distribution of phosphorus in the blood of normal rabbits and dogs

The determination of the distribution of phosphorus in the whole blood, plasma and cells according to the scheme displayed in the accompanying tables has been described in the papers from this laboratory previously cited (1, 2, 3). The fractions actually determined were the inorganic P in the whole blood and plasma, the total acid-soluble P in the whole blood, and the total P in the whole blood and plasma. The organic acid-soluble fraction, or ester P, was estimated by subtracting the inorganic P from the total acid-soluble P, and the acid-insoluble fraction, by subtracting the total acid-soluble from the total P. The values for these fractions in the cells were respectively calculated from the whole blood and plasma values by means of the total cell volume figure. For the sake of economy of blood the total acid-soluble P of the plasma was not determined in most of the blood samples from rabbits taken in the experiments reported here. Since the value for ester P in the plasma of normal blood is usually less than 0.5 mgm. per cent, when this fraction was not actually determined, the ester P of the plasma was neglected in calculating the ester P content of the cells. Somewhat greater variability has been found among " normal " values for the ester P content of the blood cells of rabbits than of other animals, but repeated blood samples from individual rabbits have indicated that under unchanging normal conditions this value in any given animal remains fairly constant over long periods of time.

The mean values and standard deviations for the distribution of phosphorus as well as other constituents of the blood, as determined in a series of normal rabbits and dogs in this laboratory during the past four years, are listed in the first columns of Tables I and II.

TABLE I

Bilateral ligation of the ureters in rabbits. Analyses of blood samples (1) from normal rabbits, (2) from two rabbits, 5½ days after bilateral ligation of their ureters

Blood constituents	(1) Normal rabbits				(2) Rabbits with ureters ligated 5½ days	
	Number of animals	Mean	± P.E.	Standard deviation ± P.E.	Number 365	Number 388
Blood cells, total..................volumes per cent	55	38.3	± 0.35	3.84 ± 0.25	31.4	28.3
Blood cells, RBC..................volumes per cent	55	37.7	± 0.36	4.00 ± 0.26	30.2	27.1
Erythrocyte count................millions per c.mm.	55	6.409	± 0.068	0.750 ± 0.048	4.40	3.8
Erythrocyte size....................cubic microns	55	68.1	± 0.54	5.99 ± 0.39	68.6	71.4
Hemoglobin, whole blood..........grams per 100 cc.	55	12.8	± 0.15	1.64 ± 0.11	8.6	8.0
Hemoglobin, cells................grams per 100 cc.	55	34.0	± 0.20	2.15 ± 0.14	28.8	29.6
Hb : RBC count ratio	55	2.32	± 0.02	0.26 ± 0.02	1.95	2.11
Nonprotein nitrogen..............mgm. per 100 cc.	39	29.7	± 0.72	6.69 ± 0.51	297.0	231.0
Chloride, plasma..................m.M. per liter	29	102.5	± 0.56	4.49 ± 0.40	72.0	78.0
Chloride, cells....................m.M. per liter	29	53.6	± 0.89	7.11 ± 0.63	43.0	46.0
Phosphorus distribution						
Whole blood						
Inorganic........................mgm. per 100 cc.	64	3.81	± 0.07	0.78 ± 0.05	18.2	14.2
Total acid-soluble................mgm. per 100 cc.	64				52.6	46.1
Ester P..........................mgm. per 100 cc.		30.6	± 0.43	5.07 ± 0.30	34.4	31.9
Total............................mgm. per 100 cc.	38	42.0	± 0.56	5.10 ± 0.39	66.7	59.1
Acid-insoluble....................mgm. per 100 cc.					14.1	13.0
Ester P : RBC count ratio	54	5.70	± 0.07	0.78 ± 0.05	7.64	8.39
Plasma						
Inorganic........................mgm. per 100 cc.	44	3.79	± 0.08	0.83 ± 0.06	20.6	16.9
Total acid-soluble................mgm. per 100 cc.					21.7	18.5
Ester P..........................mgm. per 100 cc.					1.1	1.6
Total............................mgm. per 100 cc.	37	9.1	± 0.19	1.69 ± 0.13	33.9	29.3
Acid-insoluble....................mgm. per 100 cc.					12.2	10.8
Cells						
Inorganic........................mgm. per 100 cc.	44	3.17	± 0.08	0.81 ± 0.06	13.1	7.4
Ester P..........................mgm. per 100 cc.	64	80.8	± 0.77	9.11 ± 0.54	107.0	108.7
Total............................mgm. per 100 cc.	37	97.5	± 0.86	7.76 ± 0.61	138.3	134.4

TABLE II

Bilateral ligation of the ureters in dogs. Analyses of blood samples: (1) from normal dogs, (2) from two dogs, 3 days after bilateral ligation of their ureters

Blood constituents	(1) Normal dogs			(2) Dogs with ureters ligated 3 days	
	Number of animals	Mean ± P.E.	Standard deviation ± P.E.	Number 259	Number 261
Blood cells, total ....volumes per cent	50	46.9 ± 0.40	4.20 ± 0.28	44.1	45.4
Blood cells, RBC ....volumes per cent	50	45.6 ± 0.40	4.22 ± 0.28	42.0	43.1
Erythrocyte count ....millions per c.mm.	50	6.868 ± 0.073	0.765 ± 0.052	6.47	6.35
Erythrocyte size ....cubic microns	50	66.6 ± 0.46	4.81 ± 0.32	64.9	67.8
Hemoglobin, whole blood ......grams per 100 cc.	43	16.0 ± 0.20	1.95 ± 0.14	15.21	14.94
Hemoglobin, cells ....grams per 100 cc.	43	34.9 ± 0.20	1.99 ± 0.14	36.2	34.6
Hb : RBC count ratio	43	2.36 ± 0.02	0.19 ± 0.01	2.34	2.35
Nonprotein nitrogen...........mgm. per 100 cc.	33	34.8 ± 1.15	9.78 ± 0.81	192.0	185.0
CO_2 content, plasma..........mM. per liter	34	22.2 ± 0.39	3.35 ± 0.27	19.0	26.1
CO_2 content, cells............mM. per liter	31	13.6 ± 0.28	2.30 ± 0.20	11.5	14.9
Chloride, plasma ....mM. per liter	44	105.0 ± 0.52	5.15 ± 0.37	92.5	87.0
Chloride, cells ....mM. per liter	40	56.1 ± 0.55	5.14 ± 0.39	40.3	42.9
Phosphorus distribution **Whole blood**					
Inorganic.....................mgm. per 100 cc.	38	3.03 ± 0.09	0.83 ± 0.06	9.3	15.7
Total acid-soluble.............mgm. per 100 cc.				41.2	54.4
Ester P ....mgm. per 100 cc.	38	24.6 ± 0.35	3.20 ± 0.25	31.9	38.7
Total.........................mgm. per 100 cc.	39	43.1 ± 0.51	4.72 ± 0.36	58.0	74.8
Acid-insoluble................mgm. per 100 cc.	38	15.6 ± 0.25	2.29 ± 0.18	16.8	20.4
Ester P : RBC count ratio	38	3.45 ± 0.04	0.39 ± 0.03	4.93	6.09
Plasma					
Inorganic.....................mgm. per 100 cc.	32	3.77 ± 0.14	1.20 ± 0.10	12.3	22.5
Total acid-soluble.............mgm. per 100 cc.				12.5	23.0
Ester P ....mgm. per 100 cc.	25	0.32 ± 0.03	0.20 ± 0.02	0.2	0.5
Total.........................mgm. per 100 cc.	39	16.2 ± 0.34	3.18 ± 0.24	28.2	39.6
Acid-insoluble................mgm. per 100 cc.				15.7	16.6
Cells					
Inorganic.....................mgm. per 100 cc.	32	2.25 ± 0.07	0.58 ± 0.05	5.5	7.5
Ester P ....mgm. per 100 cc.	38	51.0 ± 0.54	4.97 ± 0.38	72.1	84.6
Total.........................mgm. per 100 cc.	39	72.5 ± 0.66	6.12 ± 0.47	95.8	117.1
Acid-insoluble................mgm. per 100 cc.				18.2	25.0

Bilateral ligation of the ureters

Experimental ligation of the ureters in animals has been used many times as a means of demonstrating effects of abrupt suppression of renal function upon various constituents of the blood. Among such effects, it has been demonstrated repeatedly that increases of nonprotein nitrogen and the inorganic phosphates of the blood are closely comparable as measures of the degree of retention of waste metabolites that results from acute suppression of renal secretion. Complete studies of changes in the electrolytes of the blood serum of dogs following ureter ligation have been reported by Atchley and Benedict (8).   ·

Ureter ligation in rabbits. Under ether anesthesia and with aseptic technic, both ureters of seven rabbits were tied off by double ligatures just below the kidneys. The rabbits recovered promptly from the anesthetic and showed no immediate ill effects of the operation. Subsequently they refused food, lost weight and developed slight diarrhea. Death occurred from three to six days after the operation.

In the last two columns (part 2) of Table I are listed values determined for constituents of the bloods of two rabbits, Numbers 365 and 388, five and a half days after the bilateral ligation of their ureters. For the sake of economy of blood, complete analyses were not made in the preliminary blood samples drawn from these animals, but determinations of cell volume, red cell counts, hemoglobin, inorganic P and ester P in the whole blood sufficed to indicate that the other values, had they been determined, would have been close to the values listed for the normal. In the blood samples taken from these animals five and a half days after ligation of their ureters, the most striking changes may be noted as follows: increased nonprotein nitrogen in the whole blood; decreased chloride in both plasma and cells; increased inorganic P, greater in the plasma than in the cells; and increased ester P in the cells.

Ureter ligation in dogs. In the last two columns (part 2) of Table II are listed values determined in the bloods of two dogs, three days after bilateral ligation of their ureters. These two experiments were made at the same time as the studies of intestinal obstruction previously cited (1, 2), and the operations were performed by Dr. William DeW. Andrus. Following operation, the dogs displayed more severe and more immediate ill effects of the ligation of their ureters than did the rabbits. The dogs began to vomit about twenty-four hours after their operation and the vomiting increased in severity until the time of death which occurred on the third day, only a few hours after the final blood samples were drawn. The changes in the bloods of these dogs are similar to those found in the rabbits, namely: increased nonprotein nitrogen; increased inorganic P, higher in the plasma than in the cells; increased ester P in the cells; decreased chloride in plasma and cells. The CO_2 content of the plasma and cells remained at normal figures.

8

Mercuric chloride poisoning

Many studies, both clinical and experimental, have furnished a fairly complete picture of the chemical changes which occur in the blood plasma as a result of $HgCl_2$ poisoning. Acute nephritis with suppression of renal function is generally held to be responsible for the increase of nonprotein nitrogen and other metabolites in the blood, while vomiting and diarrhea apparently account for the depletion of the blood chlorides commonly observed (9, 10, 11). In these past investigations, however, comparatively little attention has been paid to intracellular chemical changes.

HgCl_2 poisoning in dogs. In Table III are listed the results of analyses of blood samples drawn from two dogs before and after the intravenous injection of a 1 per cent solution of $HgCl_2$. Dog Number 1, weighing 57 lbs., received 8.0 cc. of the $HgCl_2$ solution, and Dog Number 12, weighing 49 lbs., received 6.0 cc. Vomiting and diarrhea began in these dogs about ten hours after the injection of the $HgCl_2$ solution and there was rapidly progressive prostration and loss of weight. Dog Number 1 died within an hour, and Dog Number 12 about sixteen hours, after the final blood samples were drawn, these samples being taken respectively at 100 hours and forty-two hours after the $HgCl_2$ injections. Analyses of small blood samples taken between the preliminary and the last blood samples showed the progressive course of the changes in the nonprotein nitrogen, inorganic P and ester P, but the data from these analyses are not included in the table. In the final blood samples from each animal the increase of cell volume, cell count and serum protein denoted a concentration of the blood. In Dog Number 1, the size of the erythrocytes was diminished in the last sample, possibly denoting a loss of water from the cells, but in *Dog Number 12* the erythrocyte size remained practically unchanged. The slight increase in the hemoglobin: r.b.c. count ratios may indicate that new cells entered the circulation, a possibility to be considered in evaluating the chemical changes observed. The nonprotein nitrogen was markedly increased. The changes in CO_2 content and pH of the serum indicate a state of severe uncompensated acidosis. The chloride was decreased, in both plasma and cells. The inorganic P increased, more in the plasma than in the cells, and the ester P content of the cells increased. The total base of the cells changed only slightly, if at all, the changes in the observed values being within the limits of error.

Diphtheria intoxication

Among various consequences of diphtheria intoxication, the wasting of body tissues and the development of nephritis, with impairment of renal function, are responsible for a large share of the metabolic derangements which have been recognized in diphtheria. Each of these two effects has an especially important bearing upon phosphorus metabolism. In severe

TABLE III

Mercuric chloride poisoning in dogs. Analyses of blood samples taken from two dogs, before and after the intravenous injection of HgCl₂

Blood constituents	Dog Number 1		Dog Number 12	
	Before HgCl₂	100 hours after HgCl₂	Before HgCl₂	42 hours after HgCl₂
Blood cells, total....................*volumes per cent*	50.0	52.5	43.2	47.7
Blood cells, RBC.....................*volumes per cent*	49.4	51.3	42.4	46.1
Erythrocyte count.................*millions per c.mm.*	6.44	7.40	6.22	6.90
Erythrocyte size.......................*cubic microns*	76.7	69.3	68.2	66.8
Hemoglobin, whole blood...........*grams per 100 cc.*	16.0	20.1	13.7	16.37
Hemoglobin, cells..................*grams per 100 cc.*	32.4	39.2	32.3	35.5
Hb : RBC count ratio	2.48	2.72	2.20	2.37
Serum protein......................*grams per 100 cc.*	7.5	9.8	8.2	10.8
Nonprotein nitrogen................*mgm. per 100 cc.*	23.0	200.0	20.0	231.0
pH, serum..	7.44	7.18	7.38	7.17
CO₂ content, serum....................*mM. per liter*	19.5	15.5	24.6	11.7
Chloride, serum.......................*mM. per liter*	115.0	82.0	112.0	86.0
Chloride, cells........................*mM. per liter*	50.0	40.1	52.0	48.3
Total base, serum.....................*mM. per liter*	160.0	151.0	161.0	165.0
Total base, cells......................*mM. per liter*	108.0	107.0	111.0	117.0
Phosphorus distribution Whole Blood				
Inorganic........................*mgm. per 100 cc.*	2.8	8.2	2.13	20.0
Total acid-soluble................*mgm. per 100 cc.*	27.4	44.3	21.4	50.2
Ester P..........................*mgm. per 100 cc.*	24.6	36.1	19.3	30.2
Total............................*mgm. per 100 cc.*	40.7	61.2	30.2	68.4
Acid-insoluble....................*mgm. per 100 cc.*	13.3	16.9	8.8	18.2
Ester P : RBC count ratio	3.82	4.88	3.10	4.38
Plasma				
Inorganic........................*mgm. per 100 cc.*	4.2	11.1	3.03	25.2
Total acid-soluble................*mgm. per 100 cc.*		11.8		26.6
Ester P..........................*mgm. per 100 cc.*		0.7		1.4
Total............................*mgm. per 100 cc.*	13.6	28.3	12.1	44.0
Acid-insoluble....................*mgm. per 100 cc.*		16.5		17.4
Cells				
Inorganic........................*mgm. per 100 cc.*	1.4	5.6	1.4	14.3
Ester P..........................*mgm. per 100 cc.*	49.2	68.1	44.6	61.8
Total............................*mgm. per 100 cc.*	67.8	91.0	54.0	95.1
Acid-insoluble....................*mgm. per 100 cc.*		17.3		19.0

diphtheria intoxication, the wasting of body tissues necessarily increases the amounts of endogenous waste phosphates to be excreted, while the impairment of renal function interferes with the escape of those phosphates in the urine. It is to be expected, therefore, that in diphtheria intoxication there may occur a considerable disturbance of the total phosphorus metabolism of the body.

Description of the effects of diphtheria toxin upon susceptible animals, and reviews of the literature on this subject, may be found in papers by Welch and Flexner (12), Lyon (13), Rosenthal (14), Josephs (15), Mac-

Nider (16), and Yannet and Darrow (17). Decreased renal function with increases of the nonprotein nitrogenous constituents of the blood in diphtheria intoxication has been observed repeatedly. Karsner and Denis (18) demonstrated a retention of nitrogen in cats after the injection of diphtheria toxin. In a clinical study of the effects of infectious disease upon renal function, Frothingham (19) found that during the febrile stage of diphtheria there was an increased urea excretion and a decreased rate of phenolsulphonephthalein excretion by patients presenting no other evidence of acute nephritis. Reduced renal function, as measured by the rate of dye excretion, persisted after the more severe stages of the disease had passed. Wladimirowa (20) observed an increase of the organic acids in the blood of diphtheria patients. This increase of organic acids varied with the severity of the disease and, according to Martinson et al. (21), was accompanied by decreased alkali reserve and a decreased excretion of phosphorus in the urine. In early stages of clinical diphtheria and scarlet fever Markowa (22) observed a decrease in the chloride content of the whole blood.

Diphtheria intoxication in rabbits. When large preliminary samples of blood (20 cc. or more) were drawn from a rabbit, at least two weeks were allowed to elapse before that animal was injected with diphtheria toxin. In the experiments described here, small repeated doses of diluted toxin were injected by the marginal ear vein in sufficient amounts (determined by trial) to cause death in from six to ten days, the length of time necessary to produce the most striking chemical changes in the blood. The course of the intoxication was fairly uniform. For three to four days the rabbits appeared normal, ate as usual, and maintained or slightly increased their weights. Then followed a period of a few days when they ate less food and lost weight; their activity in this period, however, not being markedly reduced. After this came a period of increasing signs of severe intoxication, lasting two or three days, during which time the rabbits refused food and water and became inactive, scarcely moving from one position in the cage. Their eyes became dull, urine secretion decreased or ceased altogether, and diarrhea developed. Shortly before death they became lethargic, weak, and were unable to support themselves. The animals which received toxin Number 38M[51] died quietly without convulsions or apparent paralysis. In some cases tetanic muscular contractions could be induced by tapping the thigh. Toxin Number 390 produced much the same train of symptoms except that a few days before death a progressive paralysis developed, involving first the lower extremities, then mounting to the upper extremities. Death was preceded by dyspnea and convulsions. The gross pathologic changes found postmortem in these animals were similar to those which have been described repeatedly by others (12, 13, 16, 17).

In Table IV data from one such experiment are listed. A female Flemish red rabbit, Number 287, received by intravenous injection diphtheria toxin diluted 1 : 100 in 0.9 per cent NaCl solution, in repeated doses as follows: the first and second days, 0.2 cc.; the third day, 0.4 cc.; and on the fifth, sixth and seventh days, 0.2 cc. daily. On the tenth day after the first dose of toxin, the animal was found in a state of collapse and the

TABLE IV

Diphtheria intoxication in a rabbit. Analyses of blood samples taken before and 10 days after the intravenous injection of diphtheria toxin

Blood constituents		Before intoxication	10 days after intoxication
Blood cells, total	*volumes per cent*	40.7	37.4
Blood cells, RBC	*volumes per cent*	40.0	36.5
Erythrocyte count	*millions per c.mm.*	5.80	5.35
Erythrocyte size	*cubic microns*	69.0	68.3
Hemoglobin, whole blood	*grams per 100 cc.*	12.1	11.6
Hemoglobin, cells	*grams per 100 cc.*	30.2	31.8
Hb : RBC count ratio		2.09	2.17
Nonprotein nitrogen	*mgm. per 100 cc.*	24.0	300.0
Phosphorus distribution			
Whole blood			
Inorganic	*mgm. per 100 cc.*	3.31	17.4
Total acid-soluble	*mgm. per 100 cc.*	36.5	64.1
Ester P	*mgm. per 100 cc.*	33.2	46.7
Total	*mgm. per 100 cc.*	47.1	72.1
Acid-insoluble	*mgm. per 100 cc.*	10.6	8.0
Ester P : RBC count ratio		5.72	8.72
Plasma			
Inorganic	*mgm. per 100 cc.*	3.72	20.2
Total acid-soluble	*mgm. per 100 cc.*		22.8
Ester P	*mgm. per 100 cc.*		2.6
Total	*mgm. per 100 cc.*	10.3	28.4
Acid-insoluble	*mgm. per 100 cc.*		5.6
Cells			
Inorganic	*mgm. per 100 cc.*	2.7	12.8
Ester P	*mgm. per 100 cc.*	81.6	120.5
Total	*mgm. per 100 cc.*	100.7	145.3
Acid-insoluble	*mgm. per 100 cc.*		12.0

final blood sample was obtained from the heart just after the animal ceased to breathe, but with the heart still beating. The most striking changes to be noted in the data listed in Table IV are the increase of inorganic P, greater in the plasma than in the cells, the increase of ester P in the cells, and the increase of nonprotein nitrogen. The changes in the acid-insoluble fraction were variable in the series of similar experiments, but in most of the animals this fraction was decreased.

The progress of these changes is demonstrated by data from another experiment, listed in Table V. After a preliminary blood sample had been taken from a rabbit (Flemish red, female, Number 289), the animal was placed in a metabolism cage arranged for the collection of urine. The

volume and phophorus content of the urine were determined daily for four days, and then diluted diphtheria toxin (1 : 100 in 0.9 per cent NaCl solution) was injected into a marginal ear vein, as follows: 0.2 cc. in the first dose and 0.4 cc. forty-eight and ninety-six hours later. Small blood samples were obtained from the marginal vein of the opposite ear, on the days designated in the table, for the determination of inorganic P, ester P and nonprotein nitrogen. Determinations of cell volume, red cell counts and hemoglobin were made from these samples, but those values remained practically unchanged and are not included in this table. For five days follow-

TABLE V

Diphtheria intoxication, in a rabbit: Progressive changes in the blood and in urinary excretion

Days	Weight	1 : 100 diphtheria toxin	Non-protein nitrogen, whole blood	Inorganic P, whole blood	Ester P, cells	Urine volume	Urine P
	grams	cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. per day	mgm. per day
			27	3.33	80.0		
1	2830					100	47.6
2	2885					110	50.1
3	2760					140	50.0
4	2815		29	3.08		130	47.8
		0.2					
5	2860					120	50.3
6	2810	0.4	25	3.79	89.7	240	87.4
7	2775					lost	
8	2735	0.4				205	86.3
9	2730		41	3.65		170	44.4
10	2710					120	15.5
11	2640		108	6.67		20±	
12	2535		162	7.62	109.3	0	
13	2445		316	14.0	106.7	killed	

ing the first dose of toxin the inorganic P and nonprotein nitrogen content of the blood remained practically unchanged, while the volume and phosphorus content of the urine increased. The body weight remained fairly constant until the fifth day when, as the animal ceased to eat, it began to decrease. On the seventh day the volume of urine was approximately 20 cc., and on the eighth day the animal was anuric. On the ninth day it was extremely weak and was sacrificed after the last blood sample was drawn. It is noteworthy that the ester P in the blood cells was 89.7 mgm. per cent on the second day after the first dose of toxin, when the inorganic P and nonprotein nitrogen were still at normal levels, and on the eighth day the ester P was 109.3 mgm. per cent when the inorganic P was increased only to 7.6 mgm. per cent.

Diphtheria intoxication in dogs. In three dogs, diphtheria toxin was injected intravenously in small doses, repeated daily until the dogs developed marked signs of intoxication. The course was essentially similar to that observed in the rabbits. For a few days following the initial injection of toxin the dogs showed no ill effects. At about the fourth day they began to lose their appetites and became inactive; thereafter they became progressively weak and from twenty-four to forty-eight hours before death began to vomit. The chemical changes in the blood of the dogs were essentially like those observed in the rabbits, although not so great. The inorganic P and nonprotein nitrogen were determined in small blood samples taken daily or every second day during the period of these injections. A final large blood sample was drawn when the animal appeared to be *in extremis.*

Table VI lists data from one of these experiments. Dog Number 6, a mongrel male collie, weighing 70 lbs., received intravenous injections of

TABLE VI

Diphtheria intoxication in a dog. Analyses of blood samples taken before and on the eighth day of intoxication induced by repeated intravenous injections of small doses of diphtheria toxin

Blood constituents		Before intoxication	8th day of intoxication
Blood cells, total................	*volumes per cent*	53.5	50.2
Blood cells, RBC...............	*volumes per cent*	52.5	49.2
Erythrocyte count............	*millions per c.mm.*	6.74	6.63
Erythrocyte size..................	*cubic microns*	78.0	74.2
Hemoglobin, whole blood........	*grams per 100 cc.*	18.0	17.7
Hemoglobin, cells...............	*grams per 100 cc.*	34.3	36.1
Hb : RBC count ratio		2.67	2.68
Serum protein.................	*grams per 100 cc.*	5.9	6.5
Nonprotein nitrogen............	*mgm. per 100 cc.*	28.	182.
Chloride, serum...................	*mM. per liter*	104.0	99.0
Chloride, cells....................	*mM. per liter*	53.5	45.5
Phosphorus distribution			
Whole blood			
Inorganic.....................	*mgm. per 100 cc.*	2.34	10.3
Total acid-soluble..............	*mgm. per 100 cc.*	26.0	42.1
Ester P.......................	*mgm. per 100 cc.*	23.7	31.8
Total........................	*mgm. per 100 cc.*	41.0	56.8
Acid-insoluble.................	*mgm. per 100 cc.*	15.0	14.7
Ester P : RBC count ratio		3.51	4.72
Plasma			
Inorganic.....................	*mgm. per 100 cc.*	2.90	12.8
Total acid-soluble..............	*mgm. per 100 cc.*	3.1	13.9
Ester P.......................	*mgm. per 100 cc.*	0.2	1.1
Total........................	*mgm. per 100 cc.*	17.2	31.5
Acid-insoluble.................	*mgm. per 100 cc.*	14.1	17.6
Cells			
Inorganic.....................	*mgm. per 100 cc.*	1.9	7.8
Ester P.......................	*mgm. per 100 cc.*	44.1	62.3
Total........................	*mgm. per 100 cc.*	61.7	81.9
Acid-insoluble.................	*mgm. per 100 cc.*	15.7	11.8

diphtheria toxin Number 38M[51] diluted 1:100 in 0.9 per cent NaCl solution, as follows: the first, second and third days, 2.0 cc.; the fourth day, 3.0 cc.; the fifth day 5.0 cc.; and the sixth day, 4.0 cc. The final blood sample was drawn on the eighth day of intoxication, when the animal looked as if he would not survive another day. In this blood sample the inorganic P was increased, more in the plasma than in the cells, and the ester P was increased in the cells. The acid-insoluble P of the cells increased in one of the three dogs injected in this manner, and decreased in the other two. The nonprotein nitrogen was markedly increased, and the chloride content of both plasma and cells decreased, in all three dogs. The red blood cell counts, cell volume, erythrocyte size and hemoglobin values did not change notably during the period of intoxication.

Control studies

To ascertain what effects the loss of blood alone, in amounts such as those taken for analysis in these experiments, might have upon the distribution of phosphorus in the blood, analyses were made of blood samples from normal rabbits weighing from two to three kilograms from which different amounts of blood had been taken at varying intervals. The results of these experiments may be summarized briefly as follows. About twenty-four hours after the withdrawal of from 15 to 25 cc. of blood, there was found a slight increase, as much as 5 mgm. per cent, in the ester P content of the blood cells, but two weeks after such a blood sample was drawn the cell values were practically the same as in the first sample. Repeated bleedings at short intervals resulted in greater increases in the ester P content of the cells. For example: From one rabbit weighing 3.5 kgm., 30 cc. of blood were drawn from the heart, and at twenty-four, seventy-two and ninety-six hours thereafter repeated samples of 20 cc. each were drawn. In the successive blood samples the red cell count fell from 5.4 millions to 3.98 millions per c.mm., and the ester P content of the cells increased progressively from 85.4 to 95.0 mgm. per cent. These amounts of blood were, of course, considerably in excess of the amounts taken in the experiments with diphtheria intoxication and ureter ligation.

Because the rabbits refused food as symptoms of intoxication developed, the effects of deprivation of food on the blood phosphorus distribution were studied in three normal rabbits. In the bloods of these three animals deprived of food for 72, 96 and 120 hours respectively, the inorganic P remained unchanged, the nonprotein nitrogen increased slightly (average, from 25 to 38 mgm. per cent), and the ester P content of the cells decreased (average, from 82 to 71 mgm. per cent). Similar results of deprivation of food in dogs were described in a previous paper (1).

DISCUSSION

Increase of inorganic phosphates in the blood in severe nephritis is commonly regarded, like increase of the nonprotein nitrogen, as a sign of im-

paired kidney function; such increase being due, apparently, mainly to a failure of excretion of those endogenous waste products which ordinarily leave the body in the urine. In past investigations of chemical changes of the blood in nephritis due to different causes, considerable attention has been paid to increases of inorganic phosphates with regard to their physiologic effects and to their significance in prognosis. DeWesselow (23), for example, claimed that in nephritis a concentration of 8 mgm. per cent of inorganic phosphorus in the plasma indicated an early fatal termination, and that the level of phosphate had a greater significance in prognosis than that of the nonprotein nitrogenous constituents of the blood. The relative importance of such increases of inorganic phosphates in the total electrolyte equilibrium of the blood in nephritis has been the subject of some debate. Marriott and Howland (24) maintained that the retention of waste endogenous phosphates in the blood was largely responsible for nephritic acidosis. On the other hand, Peters and his associates (25) later presented evidence to show that in most cases of nephritis the elevation of inorganic phosphates in the blood was much less important, quantitatively, than other acidogenic factors. In this connection it should be noted that the inorganic phosphates of the blood rise highest in those circumstances where the suppression of renal function is nearly complete and fairly abrupt; it is in these circumstances, in fact, that the increases of inorganic phosphates are most often great enough to have a considerable importance in the shift of electrolyte equilibrium.

Comparatively few studies have been made of the organic acid-soluble phosphorus, or ester P, compounds of the blood in nephritis. Those that are available, however, furnish data which are in accord with the experimental findings reported here. Byrom and Kay (26) studied the distribution of phosphorus in the bloods of thirty-nine human cases of nephritis and other renal disorders of varying severity and found that in every case the ester P in the cells (designated by these authors the "P index") was increased, the figures ranging from the normal value of 53 to as high as 65. They partitioned the ester P into two fractions, respectively "hydrolyzable" and "non-hydrolyzable" by phosphatase extracted from bone, and found that in these cases of nephritis there was a change in the relative proportions of the two fractions, the hydrolyzable fraction being increased and the other decreased. Later Hoesch (27) reported studies of the distribution of phosphorus in the blood in nephritis and fractionated the ester P by hydrolysis with normal hydrochloric acid. He found the acid-hydrolyzable ester P increased in relation to the acid-non-hydrolyzable fraction. In his studies only changes in the whole blood are reported, while in the data of Byrom and Kay (as in those reported here) concentrations were expressed in relation to unit volume of packed cells. Hoesch stated, concerning a case of uremia under discussion, that if the lowered cell count in

this case were considered, both fractions of the ester P would be found quantitatively increased (in the cells).

Although the exact nature of the organic acid-soluble phosphorus compounds of the blood cells is not known, they appear to be a mixture of glycero- and hexose-phosphoric acid esters, and to be bound to part of the alkali of the cells as non-diffusible anions (28). The conditions which govern the changes of the ester P compounds in the cells, even in normal circumstances, are not well understood. The enzymatic reactions responsible for the conversion of the phosphoric esters of the blood cells are said to be extremely sensitive to changes of reaction in their medium. Martland (29) found that phosphoric ester synthesis was inhibited by a shift of pH to below 7.3, and stopped below pH 6.8, and on the other hand Rona and Iwasaki (30) found that ester hydrolysis occurred over a range pH 6.0 to 9.0. Martland suggested that the high concentration of inorganic P found in the blood in states of acidosis in many diseases might be due to the fact that a shift in the reaction of the blood towards increased acidity diminishes phosphoric ester synthesis, permitting the inorganic P to increase progressively by ester hydrolysis. Such an hypothesis appears to be inadequate, however, in view of the conditions found in the two dogs with $HgCl_2$ poisoning here described: in these animals there was a pronounced state of acidosis (serum pH 7.18 and 7.17) and yet the increases of ester P in the cells in each case were much greater than the increases of inorganic P in the plasma. It is, of course, possible that changes in concentration of intracellular ester P compounds may not follow the same course when the inorganic phosphates of the blood (those entering the blood as products of the catabolism of body tissues, or those formed as end-products of ester hydrolysis) accumulate because of failure of their removal from the blood plasma by excretion through the kidneys. As the organic phosphorus compounds increase in the cells, it may be presumed that the phosphates involved in the synthesis of those compounds are drawn largely from the plasma. Aside from the question of how the ester synthesis is brought about, however, it seems possible that the interchange between the inorganic phosphates of the plasma and the ester P of the cells may serve an important function in maintenance of the acid-base balance of the whole blood.

Hypochloremia commonly occurs in the terminal stages of severe nephritis, but the various factors influencing its development are not always clear. The decrease of blood chlorides found following $HgCl_2$ poisoning in dogs has been demonstrated by Trusler, Fisher and Richardson (10) to be due mainly to losses of Cl from the body by vomiting and diarrhea. When found thus accompanying severe vomiting, the hypochloremia of severe nephritis appears to be easily explained, although some investigators have claimed that in many cases of nephritis the loss of Cl by vomiting is insufficient to account for the decrease of blood chlorides (31). Whatever

may be the mechanism of such losses in different cases of nephritis, however, the decrease of Cl in the blood is often great enough to constitute a noteworthy disturbance which necessitates compensatory shifts in other electrolytes. Such chemical adjustments in the blood serum are well illustrated in the experiments of Atchley and Benedict (8) on the effects of bilateral ligation of the ureters in dogs: in those animals, after ureteral ligation there was found in the blood serum an average decrease of chloride plus carbonates amounting to 23.4 m. Eq., and an average increase of inorganic phosphates plus sulphates amounting to 22.3 m. Eq., practically an equimolecular interchange. The alterations of serum and cell chlorides in most pathologic conditions follow approximately parallel courses (32, 33, 34), but the compensating chemical adjustments which must occur within the cells have not received as much attention as have the changes which occur in the serum.

In earlier papers (1, 2) from this laboratory which dealt with the effects of high intestinal obstruction, evidence was presented which indicated that a reciprocal relationship existed between alterations of Cl and ester P within the blood cells. In dogs with high intestinal obstruction, it was found that, concomitant with the lowering of blood chlorides which resulted from vomiting, there was a progressive increase of the ester P concentration in the blood cells; and it appeared likely that as these organic P compounds increased they were bound to the alkali in the cells from which Cl was lost. Such a relationship between these compounds is again suggested by the observations made following the abrupt suppression of renal function by ligation of the ureters and by HgCl₂ poisoning and diphtheria intoxication, as just described. In these conditions a similar inverse relationship is apparent in the increases of ester P and the decreases of Cl in the blood cells.

SUMMARY

Studies are reported of changes in the distribution of phosphorus and other chemical constituents of the bloods of rabbits and dogs subjected to three experimental procedures (intravenous injections of diphtheria toxin and of HgCl₂, and bilateral ligation of the ureters) which result in acute suppression of renal function.

Attention is directed principally to the following changes demonstrated in the blood: 1—increased inorganic phosphates, greater in the plasma than in the cells; 2—increased organic acid-soluble phosphorus (ester P) in the cells; 3—decreased Cl in both plasma and cells; 4—increased nonprotein nitrogen.

These observations suggest that an interchange between inorganic phosphates of the plasma and phosphoric esters of the cells may play a part in adjustments of the acid-base balance of the whole blood. The findings are cited as further evidence supporting the hypothesis, previously offered,

that a reciprocal relationship exists between chloride and phosphoric esters as anions bound to alkali in the cells.

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LIPOIDS OF SERUM IN DIABETIC ACIDOSIS

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It has been pointed out in previous papers from this department (36, 42, 43) that dehydration is largely responsible for the clinical symptoms of diabetic acidosis and that the progress of recovery is correlated with the restoration of the salt and water content of the body. In the present study of diabetic acidosis, changes in lipemia have been compared with variations in serum protein concentration for the double reason that not only are proteins indicative of the course of clinical improvement (42), but also that alterations in hemoconcentration should produce marked differences in the plasma lipoids if normal capillaries are, as has been demonstrated, equally impermeable to proteins and lipoids (40). In every instance, it has been found that the elevation of serum proteins at the height of acidosis is accompanied by an increase of serum non-phospholipoid fatty acids, lipoid phosphorus and cholesterol, and that during recovery the concentrations in blood serum of all four of these constituents decrease rapidly. In an attempt to correlate the interrelations between these substances, the independence of variations in cholesterol and non-phospholipoid fatty acids and the similarity in behavior of proteins and cholesterol have become apparent in patients both with and without diabetic acidosis.

MATERIALS AND METHODS

In fourteen patients who survived, and in one who died in acute circulatory collapse, blood serum has been examined at frequent intervals during recovery from diabetic acidosis. The duration of each study varied with the individual, but was usually thirty-six to forty-eight hours, the last blood being taken twelve to twenty-four hours after the disappearance of clinical symptoms of coma or acidosis. Blood sugar, serum bicarbonate, proteins, non-phospholipoid fatty acids, lipoid phosphorus, and in 10 cases cholesterol were determined by methods of analysis described in earlier papers from this department (2, 13, 37, 39, 44, 53). Total acid-base studies were made on four of the patients. In addition, serum lipoids and proteins from certain patients without diabetic acidosis were compared.

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Non-phospholipoid fatty acids were estimated by subtracting from the titrated fatty acids 82 per cent of the fatty acids in phosphatides, in which it has been assumed that one combining weight of phosphorus unites with two of fatty acids (37). The non-phospholipoid fatty acid values are subject, therefore, to the cumulative errors of the measurements of lipoid phosphorus and fatty acid. The errors of the lipoid methods have been discussed previously (37, 39). Since the magnitude of the differences between duplicates does not usually approach the maximum error, the deviation of each determination from the mean of the duplicates has been calculated for all data included here. The average deviation of the 73 cholesterol determinations was 3.7 mgm. or the percentage deviation of each single determination from the mean of the duplicates was 1.46 per cent. Of the eighty-four determinations of fatty acids the average deviation was 0.26 m. Eq. and the percentage deviation was 1.20 per cent. Eighty-one determinations of lipoid phosphorus were made with an average deviation of 0.19 mgm. and a percentage deviation of 1.49 per cent. The error in the estimation of non-phospholipoid fatty acids is not the sum of the errors of the lipoid phosphorus and fatty acid methods because the lipoid phosphorus value used to calculate the phosphatide fatty acids involves only a fraction of the titrated fatty acids. Although the value of this fraction may vary with the individual, the average of twelve fatty acid determinations on eleven normal subjects (38) was 46.3 per cent of the average titrated fatty acids. For this reason only about half of the error of the lipoid phosphorus method enters into the estimation of non-phospholipoid fatty acids.

<center>DATA</center>

In Figure 1 alterations of serum proteins, non-phospholipoid fatty acids, lipoid phosphorus and cholesterol during recovery from diabetic acidosis are represented on a percentage basis, assuming that the initial concentration of each serum constituent is 100 per cent.

In Table I are presented the actual data from which these percentage curves were calculated, together with relevant features of treatment and records of blood sugar and serum bicarbonate. In the second column under each lipoid fraction, by comparison with the serum proteins, is given the concentration of the substance to be expected merely from the effects of hemoconcentration. It has been assumed in each case that the reduction of protein during recovery from acidosis was referable merely to dilution of the serum with saline water. The lowest value of protein attained in the acute stage of the condition is assumed to represent the point at which the normal water content of the serum has been restored. If the lipoids are equally affected by alterations of water, the actual amounts of these constituents in the serum must be measured by the concentrations observed at this point. By correcting earlier values in proportion to the
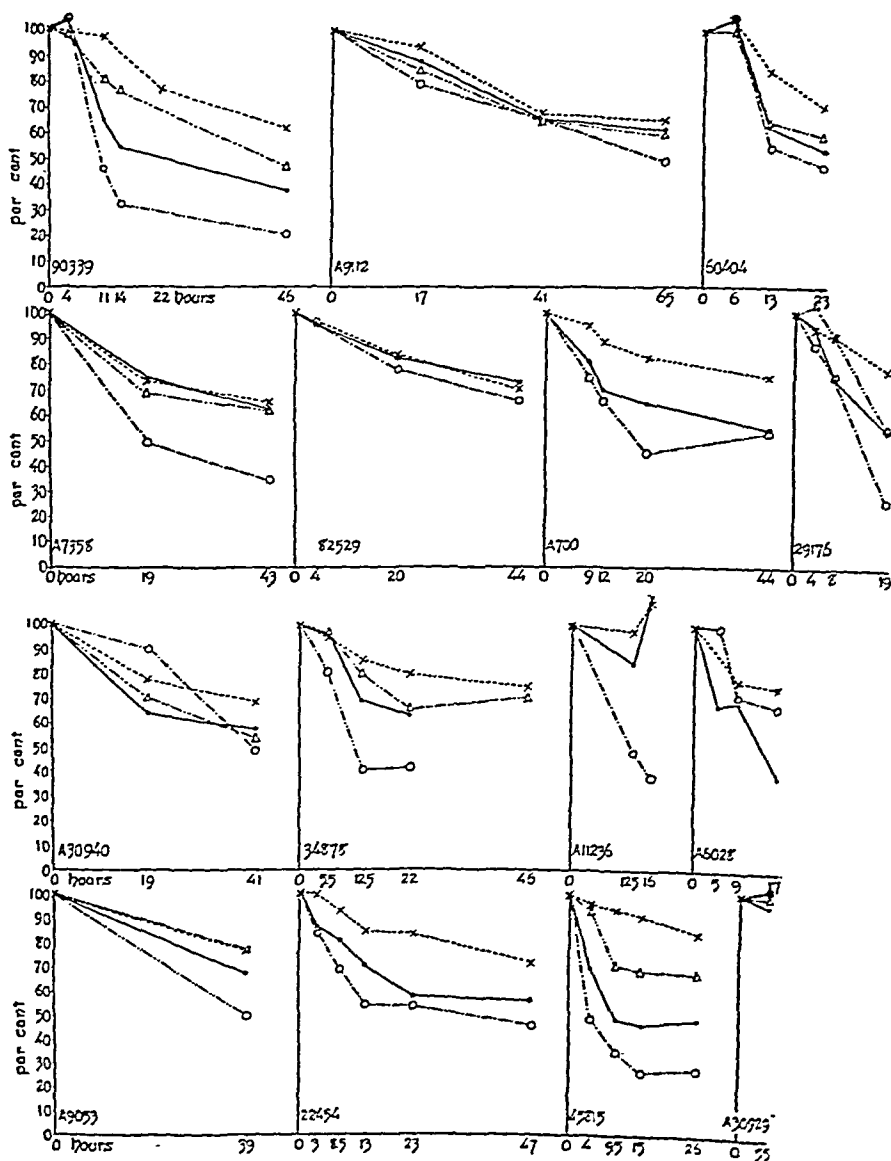
FIGURE I. RELATIVE CHANGES IN SERUM LIPOIDS AND PROTEINS DURING THE
ACUTE STAGES OF RECOVERY FROM DIABETIC ACIDOSIS

Serum proteins   x - - - x - - - x

Serum cholesterol   △ —··—··— △

Serum non-phospholipoid fatty acids   o —··—··— o

Serum lipoid phosphorus   · ——— ·

TABLE I

Changes of lipoids and proteins of serum during recovery from diabetic acidosis

Case number	Date	Time	Treatment ** Carbohydrate (grams)	Insulin units	Fluid (cc.)	NaCl (grams)	Serum Blood sugar (mgm. per 100 cc.)	Bicarbonate (m. Eq.)	Protein (per cent)	Cholesterol Observed (mgm. per 100 cc.)	Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Non-phospholipoid fatty acids Observed (m. Eq.)	Non-phospholipoid fatty acids Expected from hemoconcentration (m. Eq.)	Lipoid phosphorus Observed (mgm. per 100 cc.)	Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
82529	1932 April 2	12:15 p.m.		-			236	12.9	8.45			33.0	30.7	23.1	23.2
	April 2	4:30 p.m.	75	50	3700	20	314	12.8	7.08			31.9	25.8	22.1	19.4
	April 3	8:30 a.m.	140	130	3000†	9	71	12.9				25.7	21.6	19.1	16.3
	April 4	8:30 a.m.	105	20	1000		*400*		*5.94*			*21.6*	*21.6*	*16.3*	*16.3*
A 6028	March 22	2:00 p.m.	50	40	5000	36	413	20.8	8.98			11.5	10.2	10.1	5.3
		7:00 p.m.	45	50	825		294	19.3	6.98			11.4	8.0	6.8	4.1
		11:00 p.m.	45	60	750		277	19.5				8.2	7.7	7.0	4.0
	March 23	7:00 p.m.							*6.74*			8.2		7.7	
	October 11						238			212		6.4		9.4	

** The treatment is recorded only for the acute period in which the diet was not taken. In each instance the treatment indicated was given during the interval immediately preceding the time at which it is reported.
† The fluid intake is only approximate because the patient vomited.

TABLE I (continued)

Case number	Date	Time	Carbohydrate (grams)	Insulin units	Fluid (cc.)	NaCl (grams)	Blood sugar (mgm. per 100 cc.)	Bicarbonate (m. Eq.)	Protein (per cent)	Cholesterol Observed (mgm. per 100 cc.)	Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Non-phospholipid fatty acids Observed (m. Eq.)	Non-phospholipid fatty acids Expected from hemoconcentration (m. Eq.)	Lipoid phosphorus Observed (mgm. per 100 cc.)	Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
A 9112	1933														
	January 8	3:15 p.m.					392	10.1	8.89	334	307	14.7	11.1	16.4	15.6
	January 9	8:00 a.m.	118	145	5750†	27	144	14.4	8.32	280	288	11.6	10.4	14.4	14.6
	January 10	8:00 a.m.		115			277	20.4	6.04	215	209	9.6	7.6	10.7	10.6
	January 11	8:00 a.m.	121	115			190	25.4	5.76	199	199	7.2	7.2	10.1	10.1
	January 16	8:00 a.m.					240		7.29	213	252	8.4	9.1	11.6	12.8
	January 26	8:00 a.m.					210		6.89	272	238	10.5	8.6	13.1	12.1
	February 1	8:00 a.m.					232			270		8.8		13.5	
A 30940	July 1	2:30 p.m.	145	180	4300	26.1	1080	11.3	7.36	157	125	6.1	4.4	8.9	7.5
	July 2	9:30 a.m.	57	20			94	19.6	5.70	110	97	5.5	3.4	5.7	5.8
	July 3	7:30 a.m.							5.01	85	85	3.0	3.0	5.1	5.1
	July 11	8:00 a.m.					321		5.33	138	90	8.1	3.2	7.9	5.4
	July 19	8:00 a.m.					381		6.03	154	102	7.2	3.6	8.8	6.1
	September 12	8:00 a.m.					273		6.51	204	110	6.8	3.9	8.5	6.6
	September 29	8:00 a.m.					321		7.29	220	124	7.6	4.4	10.5	7.4

TABLE I (continued)

SERUM LIPOIDS IN DIABETIC ACIDOSIS

Case number	Date	Time	Treatment ** Carbohydrate (grams)	Insulin units	Fluid (cc.)	NaCl (grams)	Blood sugar (mgm. per 100 cc.)	Bicarbonate (m. Eq.)	Protein (per cent)	Serum Cholesterol Observed (mgm. per 100 cc.)	Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Non-phospholipoid fatty acids Observed (m. Eq.)	Non-phospholipoid fatty acids Expected from hemoconcentration (m. Eq.)	Lipoid phosphorus Observed (mgm. per 100 cc.)	Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
A 7358	1933 September 13	12 noon	195	190	5850	23.5	756	14.3	8.56	267	250	25.6	13.3	12.8	12.1
	September 14	7:00 a.m.		40			96	24.2	6.31	184	184	12.6	9.8	9.5	8.9
	September 15	8:00 a.m.					227		5.66	165	165	8.8	8.8	8.0	8.0
	September 23	8:00 a.m.					355			276	206	11.8	11.0	12.1	10.0
	September 28	9:00 a.m.					325			304	224	11.3	16.6	13.1	11.5
A 9053	1932 December 31	5:30 p.m.	85	130	4900†	20.3	278	14.1	7.07	222		25.5	13.0	13.0	8.8
	1933 January 1	5:00 a.m.		60			324	21.3	7.48			12.7	12.7	8.8	9.2
	January 2	8:00 a.m.					372					15.1	13.2	9.9	
	January 3	8:00 a.m.					371							10.4	
A 11236	1932 January 31	4:00 p.m.	175	7350	43		326	3.3	5.72‡		171	12.8	10.4	8.8¶	10.7
	February 1	4:30 a.m.	40	1200			369	9.0	5.95		178	6.2¶	8.8¶	11.7	10.5
	February 17	8:00 a.m.	208				269	11.7	6.87	171		4.9	11.7	10.8	11.7
	March 21	8:00 a.m.	80				171		6.72	170		8.6	10.8	6.4	
									7.51			7.5	6.4		

‡ Serum protein calculated from total N of serum without correction for nonprotein nitrogen.

¶ The lipoids were not extracted from the serum until 8 hours after the blood was drawn.

TABLE 1 (continued)

Case number	Date	Time	Treatment Carbohydrate (grams)	Treatment Insulin units	Treatment Fluid (cc.)	Treatment NaCl (grams)	Blood sugar (mgm. per 100 cc.)	Bicarbonate (m. Eq.)	Protein (per cent)	Serum — Cholesterol Observed (mgm. per 100 cc.)	Serum — Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Serum — Non-phospholipoid fatty acids Observed (m. Eq.)	Serum — Non-phospholipoid fatty acids Expected from hemoconcentration (m. Eq.)	Serum — Lipoid phosphorus Observed (mgm. per 100 cc.)	Serum — Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
45815	1932 October 17	4:30 a.m.	25	30	2000	14	713	4.8	7.28	304	245	38.0	12.9	20.9	12.2
		5:45 a.m.					476	12.4	6.94	281	233	18.2	12.3	14.5	11.6
		10:00 a.m	30	60	1650	2	168	17.6	6.77	213	227	13.0	12.0	10.0	11.3
		3:20 p.m.	30	40	650	1	60	18.0	6.57	208	220	9.9*	11.6	9.6	11.0
		8:30 p.m.	40	10	1050	2	267	19.0	6.05	203	203	*10.7*	10.7	*10.1*	10.1
	October 18	8:00 a.m.	55	20	800		486	18.3	6.21	190	209	11.3	11.0	9.5	10.4
	October 19	8:00 a.m.	125	35											
90339	October 29	11:15 a.m.	55	100	1200	5	536	5.6	7.92	363	275	51.4	16.9	22.3	13.5
		3:15 p.m.	25	60	2500	18	484	4.0		357		53.6		23.1	
		10:30 p.m.					177	10.6	7.69	292	267	23.5	16.4	14.5	13.1
	October 30	1:30 a.m.	38	20	750		207			277		16.5		12.1	
		9:20 a.m.		20			126	14.7	6.10			(20.0)§			
	October 31	9:15 a.m.	38	60	2250	14	224		*4.87*	*169*	169	*10.4*	10.4	*8.3*	8.3
	November 4	8:00 a.m.					302	17.8	5.50	142	191	9.6	11.7	8.0	9.4

* Marks time of lowest observed non-phospholipoid fatty acids.

§ Value in parenthesis represents total fatty acids and is given to permit inclusion in the calculations of the analysis of October 30, 9:20 a.m., which did not include lipoid phosphorus.

TABLE 1 (continued)

Case number	Date	Time	Treatment** Carbohydrate (grams)	Insulin units	Fluid (cc.)	NaCl (grams)	Blood sugar (mgm. per 100 cc.)	Bicarbonate (m.Eq.)	Protein (per cent)	Serum Cholesterol Observed (mgm. per 100 cc.)	Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Non-phospholipoid fatty acids Observed (m.Eq.)	Non-phospholipoid fatty acids Expected from hemoconcentration (m.Eq.)	Lipoid phosphorus Observed (mgm. per 100 cc.)	Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
34878	1933 July 25	10:00 a.m.					1108	4.7	7.23	401	332	21.9	11.4	18.5	14.7
		3:30 p.m.	65	115	2600	14	762	8.4	6.82	388	313	17.7	10.7	17.6	13.9
	July 26	10:30 p.m.	60	80	2100	13	511	18.4	6.19	324	284	9.0*	9.8	12.9	12.6
	July 27	8:15 a.m.	80	60	1200	2	141		5.75	264	264	9.1	9.1	11.7	11.7
	August 30 1932	8:00 a.m.							5.37	284	247				
		4:00 p.m.					137		5.88	296	270				
60404	December 28	10:00 a.m.					588	5.7	7.77	179	150	20.2	13.5	11.8	8.9
		3:45 p.m.	64	100	2700	15	313	8.0	8.13	179	157	21.3	14.1	12.4	9.3
		10:45 p.m.	47	45	1400	1	118	18.5	6.60	115	128	11.0	11.4	7.4	7.6
	December 29 1933	9:00 a.m.	62	25	1800	8	53	22.2	5.48	106	106	9.5*	9.5	6.3	6.3
	January 3	8:00 a.m.					425		5.63	155	109	10.3	9.8	7.5	6.5
29176	March 21††	8:00 a.m.							6.40	216	162				
	May 4	3:00 p.m.	70	140	2850	14	784	7.9	8.17	289	207	16.8	10.0	11.5	
		7:00 p.m.	20	25	430		610	12.7	7.66	299	194	38.0	12.8	?0	
		10:45 p.m.	50	40	1050		425	18.8	7.42	263	188	33.2	13		
	May 5	9:30 a.m.					34	24.4	6.40	162	162	28.6			
	May 13	8:00 a.m.					375		5.95	194					
	May 16	8:00 a.m.					446		5.87						

†† The determination was made some time before the development of [illegible] trition and his diabetes was well regulated.

TABLE I (*continued*)

Case number	Date	Time	Carbohydrate (grams)	Insulin (units)	Fluid (cc.)	NaCl (grams)	Blood sugar (mgm. per 100 cc.)	Bicarbonate (m. Eq.)	Protein (per cent)	Cholesterol Observed (mgm. per 100 cc.)	Cholesterol Expected from hemoconcentration (mgm. per 100 cc.)	Non-phospholipoid fatty acids Observed (m. Eq.)	Non-phospholipoid fatty acids Expected from hemoconcentration (m. Eq.)	Lipoid phosphorus Observed (mgm. per 100 cc.)	Lipoid phosphorus Expected from hemoconcentration (mgm. per 100 cc.)
22454	1932 July 3	9:15 a.m.	50	60	2190	14	672	8.0	8.88			16.6	11.1	15.1	11.8‖
	July 3	12:30 p.m.	40	50	1200	2	495	9.3	8.85			13.9	11.1	13.1	11.8
	July 3	5:45 p.m.	60	60	1200	1	414	11.3	8.26			11.4	10.3	12.2	11.0
	July 3	10:30 p.m.	60	35	1300		275	15.3	7.46			8.9*	9.3	10.6	9.9
	July 4	8:00 a.m.					81	17.4	7.36			8.9	9.2	8.7	9.8
	July 5	8:00 a.m.					436		*6.31*			*7.9*	*7.9*	*8.4*	*8.4*
A 700	1931 December 31	11:50 a.m.	90	140	3880	23	1284	3.6	7.78†			29.6¶	21.1	23.3¶	17.0
	December 31	8:30 p.m.	20	30	800	4	686	12.3	7.38†			22.0	20.0	18.9	16.2
	December 31	11:30 p.m.					480	22.3	6.91†			19.4	18.7	16.3	15.1
	1932 January 1	8:00 a.m.	100	80	1200	2	104		6.39†			13.3*	17.3	15.0	14.0
	January 2	8:00 a.m.					375		*5.80†*			*15.7*	*15.7*	*12.7*	*12.7*
	January 4	8:00 a.m.										15.5		13.3	
	January 6	8:00 a.m.							6.16†			16.0	16.7	12.8	13.5
	January 11	8:00 a.m.										12.1		11.7	
	January 20	8:00 a.m.							6.49			11.7	17.6	13.7	14.2
A 30929	1933 July 1	1:30 a.m.	65	100	2830	18	664	11.4	6.97	454	442	13.0	13.2	13.4	12.5
	July 1	8:00 a.m.	50	75	1000	5	649		*7.12*	*452*	*452*	*13.5*	*13.5*	*12.8*	*12.8*
	July 1	1:30 p.m.					474								

‖ A single determination only was done because of insufficient material.

changes of protein it is possible to distinguish between the effects of hemo-concentration and of actual changes in the amounts of circulating lipoids. For example, in Case 82529 the protein of the serum fell, in the course of 44 hours, from 8.45 to 5.94 per cent. At the same time the non-phospholipoid fatty acids fell from 33.0 to 21.6 m. Eq. The latter value is considered to represent the concentration of lipoids in the normal volume of serum. The concentration which would have been found at the onset if the amounts of lipoids in the serum had not changed can be estimated by multiplying the observed value 21.6 by the ratio of dilution of the serum derived from the protein values, 8.45/5.94, which gives the value 30.7 m. Eq. If the assumptions are correct, the actual amount of non-phospholipoid fatty acids in the serum was not significantly greater at the height of acidosis than when recovery was complete. The 34.5 per cent fall observed during recovery, from 33.0 to 21.6 m. Eq., can be ascribed almost entirely to hemodilution. This arrangement allows the reader to estimate the true extent of the lipemia in each case and the actual quantities of lipoid removed from the serum during recovery. In some instances the proteins fell further for a few days during early convalescence. These late drops are, for reasons which have been discussed elsewhere, attributed not to restoration of the normal volume of the serum but either to excessive hemodilution or to depletion of the proteins by malnutrition (42). The point in each case which is taken to represent restoration of the normal water content of serum has been marked by italics. In certain cases the results of analyses of sera at times when the patients were not suffering from acidosis have been presented for comparison.

In Table II are shown comparisons of the changes of protein and cholesterol in the sera of a group of patients without diabetic acidosis, under various conditions which altered the water content of the blood. Simultaneous determinations of non-phospholipoid fatty acids and lipoid phosphorus, when these were made, are included in the table. In the second columns, are given the expected lipoid values for changes of blood dilution on the assumption that at the time of the first observation the serum volume was normal.

In Table III, changes of serum cholesterol and non-phospholipoid fatty acids in 7 patients without acidosis are compared. The serum lipoids and proteins from blood taken before the morning insulin or meal were determined at intervals of several days. The first column under each lipoid fraction contains the value of the determined constituent, the second column the per cent of the given value in relation to the intial concentration of the substance in blood serum. Proximity of these percentage values for cholesterol and non-phospholipoid fatty acids indicates that the changes in the two constituents are parallel; for example, in the lipemias of A30304 on May 4, 1933 and May 10, 1933; divergence of these percentage values would indicate the independence of the two lipoid fractions. The last two

TABLE II

Relation of lipoids to proteins of serum in conditions other than diabetic acidosis

Case number	Date	Protein	Serum Cholesterol		Serum Non-phospholipid fatty acids		Serum Lipoid phosphorus		Diagnosis and remarks
			Observed	Expected from hemoconcentration*	Observed	Expected from hemoconcentration*	Observed	Expected from hemoconcentration*	
		per cent	*mgm per 100 cc*	*mgm per 100 cc*	*m. Eq.*	*m. Eq.*	*mgm per 100 cc*	*mgm per 100 cc*	
A 8513	1932 December 20	6.50	326		56.2		16.0		Diabetes. Before insulin and breakfast
		7.19	375	361	56.5	62.2	16.9†	17.7	3 hours after breakfast
Pl.	1933 March 22	6.53	218	240	12.4	13.1	15.8	16.7	Normal. Before and after taking 180 grams of urea in course of March 22
	March 23	7.19	245						
A 30304	May 10	5.59	465						Diabetes. Before insulin
		5.89	497	490	11.8		16.9	16.3	1 hour after insulin
		5.76	477	479	12.9	12.8	16.7		3 hours after breakfast
91497	February 21	6.43	137	144	5.4	5.7	7.4	7.8	Dehydration from vomiting
	February 22	6.77	146		6.4		8.6		
83896	March 3	6.23	226				11.8		Diabetes. Before insulin
		6.63	244	241			12.5	12.6	1 hour after insulin

* The expected values have been calculated upon the assumption that the initial protein represented the normal degree of hemoconcentration.

† Only a single determination was made because of insufficient material.

TABLE II (continued)

Case number	Date	Protein	Cholesterol		Non-phospholipoid fatty acids		Lipoid phosphorus		Diagnosis and remarks
			Observed	Expected from hemoconcentration*	Observed	Expected from hemoconcentration*	Observed	Expected from hemoconcentration*	
		per cent	mgm. per 100 cc.	mgm. per 100 cc.	m. Eq.	m. Eq.	mgm. per 100 cc.	mgm. per 100 cc.	
A 25005	June 5	3.24	194		8.7		8.8		Nephrosis. Before acacia injection
	June 8	2.43	150	146	6.7	6.5	7.4	6.6	After acacia injection
A 31941	October 23	8.72	217		7.3		10.2		Dehydration from vomiting
	October 25	6.09	135	151	7.6	5.1	7.1	7.1	After treatment
A 9053	March 16	6.91	490		41.0		19.4		Diabetes, acromegaly, 12:15 a.m.
		6.53	515	463	38.3	38.7	18.5	18.3	6:45 a.m.
A 10517	March 31	7.13	290		17.6		12.9		Diabetes before insulin
		7.34	272	298	18.2	18.1	11.8	13.3	1 hour after insulin
A 9754	March 11	6.34	219		20.5		17.8		Emaciated female of 73. Bronchopneumonia, enlarged liver, anemia, jaundice, arteriosclerosis. Death occurred March 15
	March 13	5.60	222	193	22.5	18.1	18.6	15.7	

TABLE III

Relation of lipoids to proteins of serum in a variety of conditions. (Unless otherwise stated examinations were made in the post-absorptive state)

Case number	Date	Serum								Diagnosis and remarks
		Protein		Cholesterol		Non-phospholipid fatty acids		Lipoid phosphorus		
		Observed	As per cent of initial value	Observed	As per cent of initial value	Observed	As per cent of initial value	Observed	As per cent of initial value	
		per cent	*per cent*	*mgm. per 100 cc.*	*per cent*	*m. Eq.*	*per cent*	*mgm. per 100 cc.*	*per cent*	
	1933									
A 30304	May 4	5.81		615		15.4		21.1		Diabetes
	May 10	5.59	96	464	75	12.4	81	15.8	75	
	May 19	5.90	102	371	60	11.3	73	18.0	85	
A 30940	July 11	5.33		138		8.2		7.9		Diabetes
	July 19	6.03	113	154	112	7.2	88	8.8	111	
	September 12	6.51	122	204	148	6.7	82	8.4	107	
A 31035	July 14	7.00		244		16.9		11.0		Diabetes
	July 21	6.76	97	261	107	9.0	53	10.1	92	
A 25134	May 3	3.67		561		18.9		19.0		Nephrosis
	May 16	3.80	104	682	122	18.4	97	20.8	110	
	May 22	3.54	97	792	141	23.1	122	24.5	129	
	July 11	2.75	75	685	122	52.5	278	23.6	124	
	October 20	3.08	84	572	102	49.3	261	20.4	107	
A 9053	January 5	6.33		194		19.7		10.4		Diabetes. Before insulin
		6.02	95	192	99	28.2	143	12.0	115	3 hours after breakfast containing about 50 grams of fat
29176	May 13	5.23		182		13.8		10.1		Diabetes, 12 midnight
		5.95	114	194	107	10.4	75	9.8	97	7.30 a.m.
	May 16	5.87		197		10.9		10.4		Before insulin
		6.37	109	219	111	16.3	150	12.0	115	3 hours after breakfast containing 42 grams of fat

cases in the table illustrate the effects on the cholesterol, lipoid phosphorus and non-phospholipoid fatty acid content of blood serum of the ingestion of fat.

RESULTS

Hyperlipemia in acidosis

The concentrations of all lipoid fractions at the height of acidosis are above the subjects' normal levels, attained after recovery, as can be judged from the downward trend of the curves in Figure 1. Graphic representation permits comparison of the degree of lipemia during and after acidosis without reference to a table of the normal values for serum lipoids, which have been published previously (38, 39). Since there is great variation in post-absorptive values, even in the absence of acidosis, it is more significant to compare the lipemia of the individual during acidosis to his own level after recovery than to the lipoids of normal subjects. Examination of the actual lipoid values in Table I, instead of the curves of Figure 1, shows that at the height of acidosis the elevation of each lipoid constituent above the non-acidotic level far exceeds the errors of the experimental methods. In combination with data already published (5, 12, 47, 49, 55) this series of fourteen cases, in which serum lipoids have been determined at rather frequent intervals, presents definite evidence that the concentration of lipoids is increased at the height of acidosis, and decreases rapidly during recovery. The lipoids follow the course of serum proteins, but the reduction in the former is always equal to or greater than the fall of the latter. Furthermore, the reductions of non-phospholipoid fatty acids and lipoid phosphorus usually exceed those of cholesterol, the concentrations of which more nearly parallel the decreases of proteins.

Cholesterol during and after acidosis

The actual concentrations of cholesterol in the sera of the nine patients studied at the height of acidosis range from 157 to 454 mgm. per cent (see Table I). These values fall either in or somewhat above the range of 162 to 256 mgm. per cent, the cholesterol concentrations found in ten normal subjects in the post-absorptive state (39). Cholesterol levels immediately after acidosis were below or within normal limits, varying from 85 to 264 mgm. per cent. In every case cholesterol decreased during the 18 to 48 hour period in which symptoms of acidosis disappeared. There is a rough relation between the initial and final concentrations of cholesterol: that is, subjects with high initial values had high values after recovery and those with low values at the outset had lower final values. For example, the lowest value of 85 mgm. per cent after acidosis was found in the blood serum of the same patient (A30940) who had the lowest cholesterol, 157 mgm. per cent, when in acidosis; the cholesterol of 401

mgm. per. cent (34878) decreased only to 264 mgm. per cent, the highest value observed after recovery.

The course of cholesterol through a period of convalescence after acidosis was followed carefully in five patients, 29176, A9112, A30940, A7358, 60404. The determined values have been included in Table I. In every case cholesterol increased during convalescence, in one instance, 29176, by as little as 35 mgm. per cent, and in two instances, A30940, A7358, by as much as 135 mgm. per cent. The time at which the increase became apparent bore some relation to the nutritional state and food intakes. The correlation between these two factors and the level of serum cholesterol will be discussed elsewhere. The absence of a noticeable increase in two patients after recovery from acidosis does not invalidate the generalization that cholesterol increases during convalescence, because the serum studies in the exceptional cases were not made at appropriate intervals. Case 90339 had no increase in cholesterol four days after acidosis, at a time when hyperpyrexia and a gluteal abscess retarded convalescence. No subsequent cholesterol values were determined. The study was probably made too soon to disclose the rise of cholesterol, which did not become significant in Case A9112 until more than 5 days after recovery. The increase of the cholesterol of Case A9053 to 515 mgm. per cent two and a half months after admission is not relevant to the present discussion because, in the interval, the patient had had two operations for hyperthyroidism.

The curves in Figure 1 show that the fall of serum cholesterol was proportional to the decrease of serum proteins in three cases, A9112, A7358, A9053, but was greater than the latter by amounts exceeding experimental errors in six cases, 90339, 60404, 29176, A30940, 34878, 45815. The distinction between the two groups seems to lie in the nature and severity of the clinical condition: acidosis in the first 3 cases was mild, in the last 6, severe. In Table I the second column under cholesterol gives values expected merely as a result of alterations of serum volume. Comparison of the two columns shows that the decrease of cholesterol exceeded the effects of hemoconcentration by as much as 105 mgm. per cent in Case 29176 and by as little as 29 mgm. per cent in patient 60404. However, correction for hemoconcentration in every instance diminishes the magnitude of the observed fall. For example, the actual change in cholesterol concentration of Cases 34878 was 137 mgm. per cent, while the difference between the initial and final values after correction for hemodilution was only 68 mgm. per cent. Comparisons of the cholesterol and proteins in the serum of Case 29176 before and at the height of acidosis were possible because the subject, who had been hospitalized on March 19, 1933, for regulation of diet and insulin dosage, was readmitted in acidosis on May 4, 1933. By utilizing the March 21 values, which for convenience are included in Table I, it appears that serum proteins and cholesterol were concentrated at the height of acidosis to almost the same extent, 128 and

134 per cent respectively. That the cholesterol of serum is peculiarly subject to the influence of conditions which alter hemoconcentration is shown in Table II in which are included comparisons of proteins and lipoids in a variety of conditions other than diabetic acidosis. In the second column under cholesterol the values calculated from the initial cholesterol and the change in serum proteins agree within experimental errors with the actual determinations, except in the case of A31941 and the last three cases, which will be discussed subsequently. In the standing experiments previously cited (40) increases of serum proteins were accompanied by proportionate increases of cholesterol.

Serum non-phospholipoid fatty acids during and after acidosis

Serum non-phospholipoid fatty acids at the height of acidosis varied from 6.1 to 51.4 m. Eq. In every case except that of A30940, whose non-phospholipoid fatty acids were 6.1 m. Eq., the concentration of this lipoid fraction exceeded the range of normal variation, 5.0 to 9.4 m. Eq. (38). The levels immediately after the acute stage of acidosis varied from 3.0 to 21.6 m. Eq., but were in every instance lower than the initial values. There is no constant relationship between the initial and final concentrations of the non-phospholipoid fatty acids as can be judged by the wide variations in percentage decreases of this lipoid fraction in Figure 1. During convalescence fatty acids in the post-absorptive state increased in three patients, A30940, A7358, A11236, decreased in one, A700, and were not appreciably altered in five, A6028, A9112, 90339, 60404, and 29176. Failure to detect increases can not be attributed to the fact that the sera were examined at inappropriate intervals because some were studied after 4 and some after 12 days.

The curves in Figure 1 show proportional changes of non-phospholipoid fatty acids and proteins in only three of the fourteen cases, 82529, A6028, A9112 (of these A9112 is among those in whom cholesterol and proteins also fell proportionally; in the other two cholesterol was unfortunately not determined); in all others the reduction in non-phospholipoid fatty acids exceeded that of proteins to a variable degree. The three exceptional cases were all in mild acidosis and had been given insulin prior to admission to the hospital. In Table II the agreement between the calculated and determined values of non-phospholipoid fatty acids is not satisfactory in most instances. This indicates that, although changes in hemoconcentration undoubtedly produce variations in the concentration of this lipoid fraction, the changes during diabetic acidosis are probably referable in part to other factors.

Lipoid phosphorus during and after acidosis

Serum lipoid phosphorus at the height of acidosis varied from 8.9 to 23.3 mgm. per cent. These values fall within or above the minimum and

maximum post-absorptive levels of normal subjects, 7.1 to 11.3 per cent (39). The post-acidotic values varied from 4.0 to 16.3 mgm. per cent. During convalescence the lipoid phosphorus decreased in one instance, A11236, increased in four, A6028, A9112, A30940, and A7358, and remained the same in four, 90339, 60404, 29176 and A700. Changes of lipoid phosphorus were proportional to alterations of hemoconcentration in four instances, 82529, A11236, A9112, A7358, and exceeded decreases in serum proteins in all the remaining cases. In no instance was the decrease of lipoid phosphorus less than that of serum proteins. In Table II proportionality between protein and phospholipoid changes can not be detected because the predicted variations are within the limits of error of the method for the measurement of lipoid phosphorus.

DISCUSSION

Before the days of insulin, inability to control the onset or to accelerate relief of diabetic acidosis prevented the correlation of the lipoids of the serum with other phenomena in this condition. This may account for the failure of previous observers to reach any agreement concerning the relation of lipemia to the severity of the acidosis or the impairment of carbohydrate utilization (3, 5, 23, 28, 32, 33, 34). Now the relations between the various disturbances in the composition of the blood can be traced with greater certainty, because in most instances acidosis can be promptly relieved by the administration of insulin, carbohydrate, salt and water in proper proportions. The data here presented indicate that the concentrations in the serum of all three lipoid components were, in every case, greater at the height of acidosis than they were after recovery. They were not, however, in every case above the limits of normal variation.

Contrary to the rather generally accepted concept that the concentrations of cholesterol and fatty acids in the serum tend to run parallel courses (10, 11, 17, 30, 46, 48, 55) the changes of these two components were not, in these studies, always closely related. At the height of acidosis cholesterol was never more than 60 per cent above the upper normal limit, while the non-phospholipoid fatty acids exceeded the maximum normal value by 200 per cent in four cases and in one instance by 400 per cent. During recovery from the acute phase cholesterol diminished only 22 to 53 per cent, while the fatty acids fell 33 to 84 per cent. In the subsequent convalescence, cholesterol increased in every one of the 5 cases in which its course was adequately followed; but the fatty acids rose in only 3 of 8 cases.

It has been demonstrated that when hemoconcentration is rapidly altered cholesterol and proteins of the serum undergo proportionate changes, while the relation between fatty acids and proteins is not so constant. This is evident not only in diabetic acidosis, but also in the data of Table II and of the standing experiments on C. R. and T. K., cited in a previous

article (40). Effects of hemoconcentration may explain the rather un-expected variations in cholesterol noted by Bruger and Somach (15), Bruger and Poindexter (14) and McEachern and Gilmour (41). Exact proportionality between proteins and cholesterol could not be expected over long periods and is not found in Table III, presumably because the con-centrations of these two constituents are influenced differently by other factors than the amount of water in the serum, factors such as the state of nutrition and the amounts of cholesterol and protein in the diet. In particular instances the effect of such factors may become evident even in short intervals of time. Examples are found among the last 3 cases in Table II. In A10517 protein and cholesterol change in opposite direc-tions; however, the deviations are too small to warrant the certain ex-clusion of experimental errors. A9053, a patient with acromegalic dia-betes and symptoms suggesting involvement of the hypothalamus, for some reason had persistent hypercholesterolemia. During the interval of 6.5 hours between the analyses of serum her proteins fell distinctly, while chol-esterol rose slightly. It is reasonable to ascribe the increase of cholesterol in this case to the activity of the influences which were responsible for the initial high concentration. This cannot be attributed to hemoconcentration. The last case had signs of advanced liver disease. In view of the fre-quency with which both proteins and cholesterol of the serum are disturbed in this condition, it is not surprising to find that there is no relation be-tween the two after an interval of 48 hours.

In addition to the examples already cited, lack of parallelism between changes of cholesterol and non-phospholipoid fatty acids has been detected on several occasions in diabetic subjects without acidosis. Although in Case A30304, Table III, all the lipoid fractions diminished proportionately from May 4 to May 10, cholesterol continued to fall between the 10th and the 19th when fatty acids and lipoid phosphorus changed but little. A25434 is one of 5 patients with the nephrotic syndrome who has exhibited inverse variations of fatty acids and cholesterol in his serum. Between May 3 and May 16 his cholesterol rose while the fatty acids remained practically con-stant and between May 22 and July 11 it fell while the fatty acids increased. In Table III it may be seen that after the ingestion of approximately 50 grams of fat, variations of cholesterol are irregular, but parallel those of protein, while lipoid phosphorus and fatty acids regularly rise. These results agree with those of Hiller, Linder, Lundsgaard and Van Slyke (29), Bloor (8, 9), Knudson (35), Blix (4) and others (1, 15, 16, 22, 41, 56) in showing that cholesterol is not involved in alimentary lipemia. That cholesterol and fatty acids vary independently has already been stated by Blix (5, 6), Boyd (12) and Donomae (21).

Lipoid phosphorus in the curves of Figure 1 occupies a middle position between cholesterol and non-phospholipoid fatty acids. The concentration of lipoid phosphorus was, in the case with the greatest change, 106 per

cent higher at the height of acidosis than it was after recovery. This may be compared with 60 per cent for cholesterol and 400 per cent for fatty acids (*vide supra*). During convalescence fatty acids and lipoid phosphorus both remained essentially unchanged in 3 cases, rose together twice and fell together once; while in a single instance the fatty acids increased when lipoid phosphorus decreased and in 2 other cases the lipoid phosphorus rose when the non-phospholipoid fatty acids remained constant. The correlation between the two constituents in Table III is no more exact.

During recovery from acidosis there appears to be a fairly close parallelism between lipoid phosphorus and cholesterol. This is not maintained during convalescence, when lipoid phosphorus increases only occasionally but cholesterol almost invariably rises. In Table II the relation between cholesterol and protein is quite intimate, that between lipoid phosphorus and protein is less satisfactory. In Table III there is precise agreement between the values in the second columns under cholesterol and lipoid phosphorus in only 4 out of the first 9 studies, whereas, if the two substances varied proportionally there should be agreement in all. The reactions of lipoid phosphorus and cholesterol to fat ingestion are not similar; this was to be expected, as phospholipoid may be, and cholesterol apparently is not, involved in alimentary lipemia.

The initial concentration of cholesterol does not seem to be dependent upon the severity of acidosis. For example, of the patients who survived, 34878 had the highest cholesterol, 401 mgm. per cent, while 60404 had only 179 mgm. per cent although both were suffering from acidosis of moderate degree and exhibited during recovery increases of bicarbonate and decreases of protein in the serum of approximately the same magnitude. The cholesterol of Case A9112 in mild acidosis was 334 mgm. per cent, which does not differ greatly from the 304 and 363 mgm. per cent found in the sera of Cases 45815 and 90339 who were in a more critical condition. Case A30929 whose acidosis terminated fatally because of circulatory collapse rather than accumulation of ketone bodies and whose serum bicarbonate was decreased to only 11.4 m. Eq., had the highest cholesterol, 454 mgm. per cent, of the fifteen patients studied at the height of acidosis.

The close parallelism between the curves of cholesterol and protein during the initial period of recovery from diabetic acidosis can leave little doubt that the major portion of the cholesterol drop is referable merely to hemodilution. Some additional factor must be responsible for the fact that the fall of cholesterol so frequently exceeds that of protein by a small amount. It may be objected that parallelism of any of the lipoids with protein affords no evidence that they are affected by hemodilution on the ground that proteins cannot be used as a criterion of variations of serum concentration in conditions like diabetic acidosis in which circulatory collapse so frequently increases the permeability of the capillaries. How-

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ever, since the capillaries appear to be normally impermeable to both proteins and lipoids, the authors assume that alterations of capillary permeability would affect both similarly. It has already been mentioned that cholesterol, immediately after symptoms of acidosis and dehydration have subsided, is lower than it is at the end of convalescence. In a previous article from this department (42) it was shown that decreases of serum albumin after acidosis apparently exceed the extent and duration of the process of hemodilution. The further reduction of the protein may be referable to malnutrition. The same may well be true of the diminution of cholesterol. Unpublished data show that malnutrition may result in hypocholesterolemia, which agrees with the opinion of Gardner (27) that a low intake of cholesterol over a long period of time may lower the level of this substance in blood serum. It is quite within reason that malnutrition should affect the proteins and cholesterol of serum in different degrees. As the gradual rise during convalescence seems to attend improvement of nutrition, the loss of cholesterol, which is manifested in that part of the fall in the acute phase of recovery which exceeds the effects of hemodilution, may well be referable to the wasting influence of the acidotic condition. A similar fall of cholesterol in excess of proteins was noted in the case of A31941, Table II, a non-diabetic patient who was studied during the course of recovery from dehydration produced by vomiting. This condition like diabetic acidosis is associated with rapid wasting.

Reference to the literature throws little light on the causes of hypercholesterolemia because collateral chemical and clinical data are too scanty to permit adequate analysis of the cases and because before the advent of insulin therapy the course of cholesterolemia could not be controlled during diabetic acidosis. Donomae (21) found that cholesterol was less involved in diabetic lipemia than the other lipoid constituents of the serum. With a few exceptions the concentrations of cholesterol found in the serum in acidosis are of a magnitude compatible with the conception that hemoconcentration is the chief cause of the hypercholesterolemia (3, 23, 24, 26, 28, 32, 33, 34). In certain individual cases in early studies extraordinarily high values have been found which must obviously be attributed to other factors (23, 24). Such extreme hypercholesterolemia has not, for some reason, been encountered in any case studied in this department.

Although the concentration in the serum of non-phospholipoid fatty acids is, like that of cholesterol, affected by alterations of the water content of the serum, it seems to be controlled to a far greater degree by other influences. In all but three cases fatty acids decreased during recovery from acidosis far more than did either proteins or cholesterol. In the three exceptions (Cases 82529, A6028, A9112), in which proteins and non-phospholipoid fatty acids diminished proportionally, carbohydrate starvation had been somewhat alleviated before admission to the hospital by administration of insulin. Moreover, although cholesterol when corrected

for hemoconcentration was seldom above the normal limit at the height of acidosis and was sometimes below these limits at the end of the recovery period, non-phospholipoid fatty acids at the height of acidosis were abnormally high, and afterwards fell below normal in only one instance. Finally, during convalescence, while cholesterol gradually rose with improvement of nutrition, fatty acids more often remained unchanged or fell.

There is some reason for connecting the fatty acid increases with carbohydrate starvation which, by increasing the demand for combustion of fat, provokes a mobilization of nutritive lipoids from depots in the body. More normal metabolism may perhaps explain the absence of actual lipemia in the three exceptional cases cited above (actual is used to distinguish between the accession of lipoids to the blood and the mere concentration of lipoids already in the blood). Furthermore, the initial concentrations of fatty acids in the sera of Cases A30940 and A9053, who had also received insulin on the days on which they entered the hospital, were not as high in proportion to the concentrations after recovery as were those of the patients who, having received no insulin, were suffering from both carbohydrate starvation and dehydration. Case 82529, on admission was somewhat comatose and extremely dehydrated, but, as a result of the administration of 60 units of insulin during the preceding 24 hours, was free from ketonuria and had only moderate acidosis. From Figure 1 and Table I it may be seen that serum proteins and fatty acids paralleled one another closely during recovery. The observed and calculated initial values for fatty acids were 33.0 and 30.7 m. Eq. respectively.

That insulin has any direct effect on the fatty acids of serum is unlikely. Evidence for such a direct influence has been sought by a number of observers (18, 19, 20, 25, 31, 45, 46, 50, 51, 52, 54) with unconvincing results. Furthermore in 10 patients without acidosis no demonstrable difference was found between the lipoids of the serum before and one hour after the morning dose of insulin. There seems, therefore, to be reason to attribute the decrease of fatty acids beyond that which can be ascribed to hemodilution to the recognized effect of insulin on carbohydrate metabolism rather than to any supposititious direct effect on the mobilization or combustion of fat.

That some other factor than hemoconcentration was active in the reduction of fatty acids may be inferred from the fact that this reduction not only proceeded faster than the decrease of serum protein, but also terminated sooner. In Table I the lowest values for fatty acids are marked with asterisks. In 5 of the 7 cases with severe acidosis, 45815, 34878, 60404, 22454 and A700, the non-phospholipoid fatty acids corrected for hemodilution, reached their lowest levels while proteins were still falling.

The theory that hyperlipemia is provoked by carbohydrate starvation and represents the mobilization of nutritive lipoids to meet unusually great demands for combustion of fat has been proposed by Blix (6). F...

the introduction of insulin Blix (6) found that the lipoids in the sera of diabetic patients were higher before breakfast on mornings following fast days than they were several hours after the ingestion of bread alone or a breakfast containing 35 or 50 grams of fat. Moreover fatty acids of the serum sometimes rise during the initial stages of any fast (1, 6, 7).

Interpretation of the behavior of the lipoid phosphorus of serum during acidosis must wait upon further knowledge concerning the functions of the phosphatides. In the severer cases of acidosis, A9053, 45815, 90339, 60404, 29176 and A700, the course of lipoid phosphorus approached that of fatty acids, although it seldom decreased to as great an extent as the latter. In the milder cases it behaved more like cholesterol. This would suggest that the phospholipoids are mobilized in carbohydrate starvation only when the demand for fat is extreme; but that in milder deficiencies, for example that of Case A7358, only the fatty acids are called upon. After the ingestion of fat the lipoid phosphorus of the serum rose beyond the additive experimental errors only in those subjects in whom fatty acids increased greatly (38). That the phosphatides, like cholesterol, are influenced by the state of nutrition, is suggested by the fact that lipoid phosphorus increased in those subjects whose cholesterol increased most (A9112, A30940, A7358). All these hypotheses fail to explain why in Case A6028 lipoid phosphorus fell more than fatty acids or why there was no parallelism between these two components in A11236.

CONCLUSIONS

Cholesterol, fatty acids and lipoid phosphorus of serum have been determined at intervals during recovery from diabetic acidosis.

The concentrations of all these components fell during the acute phase of recovery.

Comparison of the lipoids with the proteins of serum indicates that a large, but variable, proportion of this diminution of lipoids is referable merely to hemodilution.

The actual increases of the lipoid content of serum caused by diabetic acidosis, therefore, appear to be much smaller than observed concentrations would indicate.

The course of cholesterol parallels that of protein more nearly than does the course of the fatty acids, while phosphatides take an intermediate position. The factors, other than hemodilution, which may be responsible for these differences are discussed.

Data from diabetic patients without acidosis and from patients with other diseases illustrate further the influence of changes of hemoconcentration upon serum lipoids.

The variations of cholesterol throughout appear to bear little relation to those of the fatty acids.

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KIDNEY FUNCTION AND BLOOD PRESSURE

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According to the filtration reabsorption theory as advanced by Ludwig (1) and by Cushny (2), and further elaborated by Rehberg (3), the hydrostatic pressure in the glomerular capillaries furnishes the potential inducing glomerular filtration. If the area of filtration membrane (i.e., the number of open glomeruli) and its permeability, and also the volume of blood passing through the kidneys per unit of time and the oxygen tension of this blood remain constant through a given length of time, the filtration (F) in the same period will be proportional to the pressure available for the filtration (T_F) :

$$F = k \times T_F = k \ (T_h - T_c - T_k),$$

where T_h is the hydrostatic pressure in the glomeruli, T_c the colloid osmotic pressure in the plasma, and T_k the hydrostatic pressure within the glomerular capsule.

The hydrostatic pressure in the glomerular capillaries cannot be measured directly, but most authors take it to be high. Ekehorn (4) estimates it at about 50 per cent of the aortic pressure, while Poulsson (5) contends it must be about 80 per cent of the aortic pressure. But even though opinions differ as to the height of the blood pressure in the kidney capillaries in relation to the aortic pressure, the structure of the capillary system of the kidneys is at any rate suggestive of a high hydrostatic pressure in the glomerular vascular skeins, as the lumen is considerably greater in the vasa afferentia than in the vasa efferentia (Ekehorn has calculated the area of the efferent vessels to be about ¼ to ⅑ of the area of the afferent vessels).

The colloid osmotic pressure in the plasma in normal persons may be set at about 30 mm. Hg, as according to Iversen and Nakazawa (6) and Ito, Seki and Nakazawa (7) the average values in normal individuals are from 360 to 385 mm. H_2O.

The hydrostatic pressure within the capsular space constitutes a direct counterpressure against filtration. It may be defined as the pressure required to force the filtrate the first part of its way down through the convoluted tubules, and consequently it will largely depend upon the functional condition of the tubules, as the main part of the reabsorption of

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water is assumed to take place in the proximal convoluted tubules. If the reabsorption of water proceeds very rapidly (i.e., when the urine volume flow is small or of moderate volume), T_k is small; and T_k increases with increasing diuresis. When the urinary output is small, therefore, T_k may be assumed to be of minor significance in the effective filtration pressure, which will then depend preponderantly upon the difference between the hydrostatic pressure in the glomerular capillaries and the colloid osmotic pressure in the plasma.

That the relation between these two pressures, T_h and T_c, is decisive with respect to the magnitude of the filtration potential has been shown experimentally by Cushny (2): the secretion of urine ceases if the blood pressure falls to about 30 mm. Hg (equal to the colloid osmotic pressure) even though the kidneys under these conditions are still supplied with about 30 per cent of their normal blood flow. Gremels and Poulsson (8) have repeated these experiments, employing the kidney-heart-lung preparation of Starling, and have found that filtration in the kidneys ceases when the blood pressure in the renal artery falls to about 30 to 40 mm. Hg. Ni and Rehberg (9) have shown that the filtration decreases when the colloid osmotic pressure increases. Finally, it may be mentioned that the marked fall of the blood pressure, which appears when the spinal cord is divided immediately below the medulla oblongata, is accompanied by a cessation of the urinary secretion that might be explained as resulting from a fall of T_h to a value below T_c.

The value of the effective filtration pressure in normal persons cannot be given with any certainty, but Krogh (10) holds that $T_h - T_c$ in the kidney capillaries is rather high, between 500 and 1000 mm. H_2O, i.e., about 40 to 80 mm. Hg.

In the clinic, the relation between glomerular filtration (estimated from creatinine clearance) and the blood pressure has been investigated to only a limited degree. Roelsen (11) has thus estimated the filtration in 25 patients suffering from essential hypertension, and only 4 of these patients showed filtration values below 100 cc. per minute; for these patients as a whole he found in the average, normal filtration values. From the data given by Roelsen, however, it appears possible that some of his patients may have had nephrosclerosis, which lesion—according to Holten and Rehberg (12)—is associated with a decrease of filtration; further, it is probable that several of these patients had cardiac insufficiency, in a greater or less degree, which also may give decrease of filtration (Lassen (14)).

Thus it cannot be said to have been shown as yet that there exists a relation between the blood pressure and glomerular filtration in man. The studies here presented were aimed to throw light on this question.

In recent years, spinal anesthesia has been employed in surgery to a large extent; as is well known, this form of anesthesia is accompanied often by a considerable fall in blood pressure. In practice this associated

effect is counteracted by intramuscular injection of ephedrine or ephetonin (0.05 to 0.1 gram) prior to the anesthetic.   If, in spite of this precaution, signs of threatening collapse appear during the operation, an increase of blood pressure may be produced almost instantaneously by placing the patient in the Trendelenburg position.   Spinal anesthesia is a conduction anesthesia, blocking sensory impulses as well as motor and vasomotor. The fall of blood pressure is due to vasodilatation, involving especially the splanchnic field, hence also the kidneys.

It seemed reasonable, therefore, to assume that patients with non-compensated fall of blood pressure after spinal anesthesia would constitute particularly suitable subjects for studies on the behaviour of filtration during variations in blood pressure.

We have examined the filtration (creatinine clearance) in 4 patients in whom the fall of blood pressure after spinal anesthesia (novocaine, 15 centigrams) was not compensated by injection of ephetonin, and we have compared the findings in these patients with those in 3 patients who were

FIG. 1.   PATIENTS NUMBER 1 TO 4 (CHARTS I TO IV)

Creatinine clearance (- - - - - -) and systolic blood pressure (x ———— x) after spinal anesthesia (indicated by arrow) without injection of blood pressure raising drugs.

water is assumed to take place in the proximal convoluted tubules. If the reabsorption of water proceeds very rapidly (i.e., when the urine volume flow is small or of moderate volume), T_k is small; and T_k increases with increasing diuresis. When the urinary output is small, therefore, T_k may be assumed to be of minor significance in the effective filtration pressure, which will then depend preponderantly upon the difference between the hydrostatic pressure in the glomerular capillaries and the colloid osmotic pressure in the plasma.

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effect is counteracted by intramuscular injection of ephedrine or ephetonin (0.05 to 0.1 gram) prior to the anesthetic. If, in spite of this precaution, signs of threatening collapse appear during the operation, an increase of blood pressure may be produced almost instantaneously by placing the patient in the Trendelenburg position. Spinal anesthesia is a conduction anesthesia, blocking sensory impulses as well as motor and vasomotor. The fall of blood pressure is due to vasodilatation, involving especially the splanchnic field, hence also the kidneys.

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We have examined the filtration (creatinine clearance) in 4 patients in whom the fall of blood pressure after spinal anesthesia (novocaine, 15 centigrams) was not compensated by injection of ephetonin, and we have compared the findings in these patients with those in 3 patients who were

FIG. 1. PATIENTS NUMBER 1 TO 4 (CHARTS I TO IV)

Creatinine clearance (- - - - - -) and systolic blood pressure (x————x) after spinal anesthesia (indicated by arrow) without injection of blood pressure raising drugs.

given an injection of ephetonin alone or of ephetonin + caffeine (caffeine sodium benzoate) prior to or coincident with the administration of the spinal anesthetic. All the patients were young men, or approaching middle age, healthy as to the condition of heart and kidneys, admitted to Department I (Surgery) of the Kommunehospital; 6 were operated on for inguinal hernia, 1 for hydrocele testis.

The technique of the creatinine method for the examination of kidney function and calculation of filtration and of the concentration index is given in detail in previous papers (Lassen (13, 14)); with due regard to the special experimental conditions in the present studies, we have in all essentials followed the method as given originally by Rehberg (15).

I. KIDNEY FUNCTION UNDER SPINAL ANESTHESIA WITHOUT EPHETONIN

Patient Number 1. S. K., 39 years.

January 16, 1932: Inguinal herniotomy under spinal anesthesia.

Time

7:00. 3 grams creatinine (Brit. Drug House) taken in 200 cc. milk.

8:00. In-lying catheter inserted. Thus all urine was obtained by catheter by evacuation from pressure on the bladder. The catheter was fixed so that its tip reached just inside the internal orifice of the urethra.

8:00. Systolic blood pressure: 130 mm. Hg. In all the experiments the systolic blood pressure alone was measured.

9:00. Spinal anesthesia. Effect: Analgesia up to the fourth rib; anesthesia up to the middle of the epigastrium. Effect ceased at 10:15.

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	*grams per liter*		*mgm. per 100 cc.*
8:00	250		0	5.10
8:30				5.00
9:00	76	4.70	0	4.23
9:30	19.5	5.60	0	4.00
10:00	5	7.70	0	3.94
10:30	6.5	10.65	trace	3.57

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure †
	cc. per minute		*cc. per minute*	*mgm. per minute*	*mm. Hg*
8:00– 9:00	123	97	1.27	5.97	130
9:00– 9:30	88	136	0.65	3.64	93
9:30–10:00	32	194	0.17	1.29	73
10:00–10:30	61	283	0.22	2.34	103

* $\dfrac{\text{Concentration of creatinine in urine}}{\text{Concentration of creatinine in blood}}$.

† Average weighted according to the intervals during the individual blood pressure readings.

Figure 1 (I) gives a graphic presentation of the relation between the systolic blood pressure and the calculated amounts of filtrate in cc. per minute.

Patient Number 2. B. J., 20 years.
April 4, 1932: Inguinal herniotomy under spinal anesthesia.
Time
7:30. 3 grams creatinine in 200 cc. milk.
In this case the bladder was evacuated spontaneously at 8:30, 9:00 and 10:00. The systolic blood pressure was measured at these times; it was constantly 120 mm. Hg. At 9:00 and 11:15, 200 cc. water were given by mouth.
10:00. Spinal anesthesia. Effect: Analgesia to fifth rib; anesthesia up to the middle of the epigastrium. Effect ceased at about 11:15.
In order to obtain a period with the blood pressure at as low a level as possible, the measurements were interrupted from 10:00 to 10:15, while the blood pressure was falling from 120 to 90 mm. Hg.
10:15. In-lying catheter inserted. The following portions of urine were obtained by catheter by evacuation from pressure on the bladder.

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	*grams per liter*		*mgm. per 100 cc.*
8:30	117		0	5.59
9:00	99	3.24	0	5.68
10:00	233	2.07	0	4.27
10:00–10:15	Measurements interrupted.			
11:15	17	12.35	0	3.85
12:15	17	12.65	0	3.25

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure †
	cc. per minute		*cc. per minute*	*mgm. per minute*	*mm. Hg*
8:30– 9:00	190	57	3.30	10.69	120
9:00–10:00	161	42	3.88	8.03	120
10:15–11:15	87	307	0.28	3.46	79
11:15–12:15	101	356	0.28	3.54	96

* $\dfrac{\text{Concentration of creatinine in urine}}{\text{Concentration of creatinine in blood}}$.
† Average weighted according to the intervals during the individual blood pressure readings.

Figure I (II) gives graphically the relation between the systolic blood pressure and the calculated amounts of filtrate in cc. per minute.

Patient Number 3. O. V. L., 50 years.

April 5, 1932: Inguinal herniotomy under spinal anesthesia.

Time

7:00. 6 grams creatinine in 250 cc. milk.

8:00. Bladder evacuated spontaneously. The patient took 200 cc. water. Systolic blood pressure 140 mm. Hg.

9:15. In-lying catheter inserted. Systolic blood pressure 140 mm. Hg. Spinal anesthesia. Effect: Analgesia to the top of the epigastrium; anesthesia up to the umbilical level. Effect ceased at about 10:30. Measurements interrupted 9:15–9:30.

10:40. The patient took 200 cc. water.

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	*grams per liter*		*mgm. per 100 cc.*
8:00	38		0	7.57
8:30				7.88
9:15	44	20.25	0	8.00
9:15–9:30		Measurements interrupted.		
10:15	3	23.89	0	7.75
11:30	16	30.63	0	6.41
12:00	10	28.20	0	5.88

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure†
	cc. per minute		*cc. per minute*	*mgm. per minute*	*mm. Hg*
8:00– 9:15	152	259	0.59	11.95	140
9:30–10:15	20	305	0.067	1.60	72
10:15–11:30	92	433	0.21	6.43	78
11:30–12:00	153	459	0.33	9.40	105

* $\dfrac{\text{Concentration of creatinine in urine}}{\text{Concentration of creatinine in blood}}$.

† Average weighted according to the intervals during the individual blood pressure readings.

Figure 1 (III) gives graphically the relation between the systolic blood pressure readings and the calculated amounts of filtrate in cc. per minute.

Patient Number 4. S. O. M., 16 years.

October 1, 1932: Inguinal herniotomy under spinal anesthesia. This patient had only one kidney, the other having been removed some years before after a traumatic injury to the lumbar region.

Time

6:00. 3 grams creatinine in 200 cc. milk.

7:00 and 8:00. Bladder evacuated spontaneously. Systolic blood pressure 130 mm. Hg.

8:15. Spinal anesthesia. Effect: Analgesia to third rib; anesthesia to the middle of the epigastrium. Effect ceased about 9:30.

8:30. In-lying catheter inserted. Blood pressure: 130 mm. Hg.
9:30. The patient took 200 cc. water.

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	grams per liter		mgm. per 100 cc.
7:00				6.41
8:00	230	2.13	0	6.17
8:00–8:30		Measurements interrupted.		
8:30				5.88
9:00	9	16.15	0	5.64
10:00	28	11.50	0	5.62
11:00	37	9.25	0	5.80

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure†
	cc. per minute		cc. per minute	mgm. per minute	mm. Hg
7:00– 8:00	130	34	3.83	8.16	130
8:30– 9:00	84	280	0.30	4.85	86
9:00–10:00	95	204	0.47	5.41	110
10:00–11:00	102	166	0.62	5.74	115

* Concentration of creatinine in urine
Concentration of creatinine in blood.
† Average weighted according to the intervals during the individual blood pressure readings.

Figure 1 (IV) gives graphically the relation between the systolic blood pressure and the calculated amounts of filtrate in cc. per minute.

RESULTS, EXPERIMENTS 1 TO 4

The results of these 4 experiments in which the filtration was followed in relation to the variations in the blood pressure which appeared under spinal anesthesia when no blood pressure raising drugs were injected, are so consistent that they may be discussed together.

The systolic blood pressure was normal before the anesthesia in all four cases. After the anesthesia was established there came a very abrupt fall in the blood pressure, the lowest level being reached within 20 to 25 minutes, except in Number 2; in this case the lowest level was not reached until 1 hour after the anesthesia was effective. In 3 of the cases, the blood pressure then rose, almost as rapidly as it had fallen, to a level somewhat lower than the initial value; the blood pressure remained at this level almost constantly throughout the remainder of the experimental period. In Number 3 the low blood pressure persisted for about 1 hour, and then rose somewhat more slowly than in the other cases.

In these four patients the minimal values for systolic blood pressure were respectively 60, 70, 70 and 75 mg. Hg, and did not appear to give the patients any inconvenience whatever. None of the patients showed any signs of threatening collapse, so that it was not found necessary in any of these cases to place the patient in Trendelenburg's position.

As we have not measured the diastolic blood pressure in these patients, we know nothing definite about the mean blood pressure which was "effective" during the various periods in which the filtration was measured. Obviously, however, it was lower than the systolic blood pressure so that it is reasonable to infer that the hydrostatic pressure in the kidney capillaries was very low during the periods when the systolic blood pressure was at its lowest level.

Filtration. In all four patients the filtration before the anesthesia was within the normal limits of variation. All four patients showed a considerable fall in the amount of filtration at the time when the blood pressure fell and, in most instances, an increase in the amounts of filtrate with increasing blood pressure. There was no strict parallelism between filtration and blood pressure in the latter part of the experiments when the effect of the spinal anesthesia was ebbing; nor is such a parallelism to be expected, we think, as the various factors effective in this period cannot be estimated. As a rule, however, the filtration increased as the blood pressure rose.

From these experiments, then, it is evident that the "kind" of variations in the blood pressure which appear during spinal anesthesia are accompanied by variations in the filtration which parallel in general the blood pressure variations and "correspond" also fairly well to them in magnitude of variation. We use the term "kind" of blood pressure variations under spinal anesthesia in order to avoid expressing any premature opinion as to the relation between blood pressure and filtration when variations of blood pressure are produced in other ways—through some other "mechanism"—than by spinal anesthesia. Probably, however, this reservation is unnecessary.

From Figure 1 it will be noticed that the filtration does not fall percentily, as much as does the blood pressure—a finding that is perfectly consistent with the formula given above for the effective filtration pressure, namely: $T_F = T_h - T_c$. (Here T_k is omitted because the pressure in the capsular space may be taken as relatively insignificant when the degree of urinary concentration is as high as it must have been during the periods of low blood pressure with which we were dealing.) The effective filtration pressure as formulated results from the difference between two values, one of which (T_c) is presumably constant throughout the experiment (about 30 mm. Hg) while the other (T_h) varies. Consequently, the effective filtration pressure must decrease percentily more than the hydrostatic pressure. (For example: if T_h falls from 100 to 50 mm. Hg, i.e.,

50 per cent, the effective filtration pressure will fall from 70 to 20 mm. Hg, that is about 70 per cent.)

The experiments do not show at what systolic blood pressure the kidney secretion ceases. In Patients Number 1 and 3, however, filtration values of 32 and 20 cc. per minute, respectively, were found corresponding to average systolic pressures of 73 and 72 mm. Hg. From this, we infer, that the systolic blood pressure in the peripheral circulation need fall but little below 70 mm. Hg before the production of urine ceases.

The *concentration index*—i.e., the ratio of creatinine concentration in the urine to creatinine concentration in the blood—may be taken to express the degree of water reabsorption in the tubules. It will be noticed that coincident with the fall of blood pressure after spinal anesthesia, and coincident with the decrease of the filtration, there was in all the cases a considerable increase in the concentration index with resulting low urine volume. In some of the cases the concentration index was even unusually high—a finding which suggests that the cells of the tubules are practically impermeable to creatinine. In contrast with the decrease in filtration, the concentration index remained high, or rose even further, as the blood pressure rose again after the primary fall. The explanation of this may be that probably the function of the tubules is regulated not alone by physicochemical factors even under conditions where conduction is blocked in all the spinal nerves up to the 6th or 7th thoracic segment (for one thing, the influence of the hypophysis is not excluded).

Urine volume flow. In these experiments, as is usually the case, the rate of urine volume flow was chiefly dependent upon the tubular function. But in the periods where the amount of filtrate per minute was small the output of urine was reduced further, which resulted in exceptionally small amounts of urine in these periods.

Here it is to be emphasized again that we have taken the greatest possible care to collect the urine as quantitatively as possible, realizing fully that when the output of urine is so small, even the slightest retention in the bladder will percentily play a very great rôle.

Summarized briefly, these 4 experiments show that in an organism in which the spinal nerves below the 6th or 7th thoracic segment are blocked by spinal anesthesia, the glomerular function of the kidneys is subject to variations which on the whole are parallel with the variations of the blood pressure, whereas the tubular function is unchanged, in the sense, that neither the conduction anesthesia *per se* nor the fall of blood pressure lowers the reabsorption of water in the tubules.

How are these findings to be explained, especially the decrease of the filtration concurrent with the spinal anesthesia and with the fall of blood pressure?

According to the filtration reabsorption theory, the decrease of filtration can readily be explained as a result of the fall of blood pressure, and the

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high concentration indices may be attributed to the scantiness of filtrate which, probably on account of the low filtration pressure, passes through the tubules more slowly than normally thus giving the tubules "time" for a hypernormal reabsorption of water.

Considered from the viewpoint of the secretion theory, on the other hand, one might interpret the small urine volume after spinal anesthesia as due to a lack of secretory impulses, because the secretory nerves are blocked. If that were the case, however, it would be necessary to assume that the conduction anesthesia blocked only the secretory nerves to the glomeruli, as it is unquestionably evident from these experiments that the tubular function is not lowered, the water reabsorption being rather above normal. In order to explain the high creatinine concentration in the urine, one would then have to resort to the auxiliary hypothesis, that under spinal anesthesia the cells of the tubular epithelium might be particularly apt to secrete creatinine—a rather improbable possibility.

So, the findings *may* be explained in accordance with either theory, although the explanation is much more simple when based on the filtration theory than when elaborated from the viewpoint of the secretion theory.

In order to throw additional light on the problem we have conducted some control experiments. In 3 patients we have examined the filtration under spinal anesthesia during which the fall of the blood pressure was counteracted by preceding or by simultaneous injection of ephetonin or of ephetonin + caffeine (caffeine sodium benzoate). The underlying idea was, that if it be shown that spinal anesthesia, fully effective in the extent of analgesia and anesthesia but without concurrent fall of the blood pressure, be not accompanied by a decrease of filtration, such a finding would make it more difficult to explain the preceding experiments in accordance with the secretion theory.

Figure 2 (V) gives graphically the relation between the systolic blood pressure and the calculated amounts of filtrate in cc. per minute.

II. KIDNEY FUNCTION UNDER SPINAL ANESTHESIA WITH SIMULTANEOUS ADMINISTRATION OF EPHETONIN

Patient Number 5. O. C. R., 24 years.

February 29, 1932: Inguinal herniotomy under spinal anesthesia.

Time

7:00. 3 grams creatinine in 200 cc. milk.

7:55. In-lying catheter inserted. Systolic blood pressure: 130 mm. Hg.

8:30. Systolic blood pressure: 120 mm. Hg.

9:20. Systolic blood pressure: 125 mm. Hg. Injection of 0.1 gram ephetonin + 0.3 gram caffeine sodium benzoate. Spinal anesthesia. Effect: Analgesia up to the top of the epigastrium; anesthesia to the umbilical level. Effect ceased at about 10:30.

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	*grams per liter*		*mgm. per 100 cc.*
7:55	135	3.80	0	4.90
8:30	43	6.44	0	4.99
9:29	61	6.56	0	4.76
10:13	56	6.36	0	4.14
11:00	· 25	9.64	0	3.60

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure†
	cc. per minute		*cc. per minute*	*mgm. per minute*	*mm. Hg*
7:55– 8:30	157	130	1.23	7.92	125
8:30– 9:29	139	134	1.03	6.76	121
9:29–10:13	182	143	1.27	8.08	124
10:13–11:00	132	249	0.53	5.11	129

* $\dfrac{\text{Concentration of creatinine in urine}}{\text{Concentration of creatinine in blood}}$

† Average weighted according to the intervals during the individual blood pressure reading.
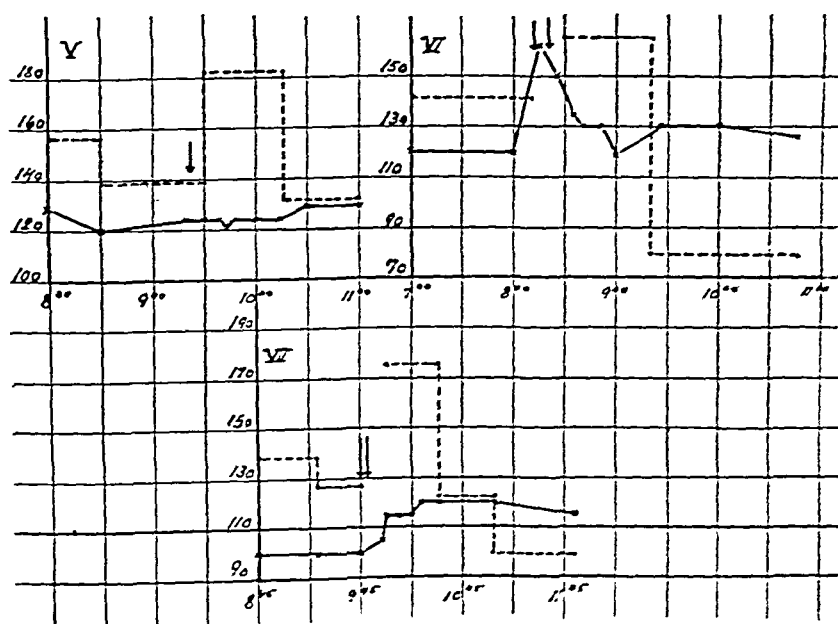
FIG. 2. PATIENTS NUMBER 5 TO 7 (CHARTS V TO VII)

Creatinine clearance (- - - - - -) and systolic blood pressure (x ——————— x) after spinal anesthesia with simultaneous injection of ephetonin (both indicated by arrows).

Chart V: 9:22. Ephetonin 0.10 gram + caffeine sodium benzoate 0.3 gram. 9:22. Spinal anesthesia.

Chart VI: 8:15. Ephetonin 0.05 gram + caffeine sodium benzoate, 0.3 gram. 8:20. Spinal anesthesia.

Chart VII: 9:45. Ephetonin 0.10 gram. 9:50. Spinal anesthesia.

Patient Number 6.   R. S., 49 years.

October 21, 1932: Operation for hydrocele (testis) under spinal anesthesia.
Time
6:00.   3 grams creatinine in 200 cc. milk.
7:00.   Bladder emptied spontaneously. Systolic blood pressure: 120 mm. Hg.
7:30.   Intake of 200 cc. water. At 8:00, systolic blood pressure: 120 mm. Hg.
8:10.   In-lying catheter inserted.
8:15.   Injected 0.05 gram ephetonin + 0.3 gram caffeine sodium benzoate.
8:20.   Spinal anesthesia. Effect: Analgesia to fourth rib; anesthesia to the
        middle of the epigastrium. Effect ceased about 9:30. Measurements
        interrupted 8:10–8:30 (as in Number 2, 3 and 4).

Time	Urine output	Creatinine in urine	Albumin in urine	Creatinine in blood
	cc.	*grams per liter*		*mgm. per 100 cc.*
7:05				6.37
8:10	88	6.95	0	
8:15				5.75
8:10–8:30		Measurements interrupted.		
9:20	96	5.00	0.45 per cent	
9:25				5.69
10:45	55	6.85	0	5.52

Time	Filtration	Concentration index*	Urine volume	Creatinine output	Average systolic blood pressure†
	cc. per minute		*cc. per minute*	*mgm. per minute*	*mm. Hg*
7:00– 8:10	143	114	1.26	8.76	122
8:30– 9:20	168	88	1.92	9.60	128
9:20–10:45	79	122	0.65	4.45	129

* Concentration of creatinine in urine
  ————————————————————
  Concentration of creatinine in blood

† Average weighted according to the intervals during the individual blood
pressure reading.

Figure 2 (VI) gives graphically the relation between the systolic blood
pressure and the calculated amounts of filtrate in cc. per minute.

Patient Number 7.   A. C., 35 years.

January 11, 1933: Inguinal herniotomy under spinal anesthesia.
Time
7:30.   3 grams creatinine in 200 cc. milk. At 8:40, systolic blood pressure:
        100 mm. Hg.
8:45.   In-lying catheter inserted.
9:20.   Intake of 200 cc. water. Systolic blood pressure: 100 mm. Hg.
9:45.   Ephetonin, 0.1 gram. Systolic blood pressure: 100 mm. Hg.
9:50.   Spinal anesthesia. Effect: Analgesia up to the nipple; anesthesia up
        to the middle of the epigastrium. Effect ceased about 11:00.
9:45–10:00.   Measurements interrupted.
11:05.   Intake of 200 cc. water.

b. At the same time, the concentration index increased significantly and remained high, or rose even further during the subsequent gradual rise of blood pressure.

Thus the tubular function was normal, as far as the reabsorption of water is concerned, or even hypernormal at the same time that the effect of the spinal anesthesia was at its height and the filtration was lowered.

c. During the fall of blood pressure there was an enormous decrease in the urine volume, partly on account of considerable reabsorption of water in the tubules, partly on account of the lowered filtration in the glomeruli.

2. In 3 control experiments the course of the glomerular filtration was followed under spinal anesthesia during which the blood pressure fall was counteracted by injection of ephetonin or ephetonin + caffeine. It was found that:

Ephetonin (or ephetonin + caffeine) was able under optimally effective spinal anesthesia to maintain at the same time both a normal blood pressure and a normal glomerular function. The function of the tubules was not affected by this.

3. The relation that was found between the blood pressure and the kidney function under the experimental conditions described can be readily explained in accordance with the filtration reabsorption theory.

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spinal anesthesia had been established, which was so conspicuous a phenomenon in Experiments 1 to 4. On the contrary, all of the Experiments 5 to 7 showed an increase of filtration during this period followed subsequently by a fall, mostly gradual, in the later periods when the anesthesia was about to subside.

The *concentration index* and *urine volume* followed about the same course, showing at any rate no significant changes during the periods when the effect of the spinal anesthesia was at its height.

The experiments show, then, that under. optimally effective spinal anesthesia the administration of ephetonin or ephetonin + caffeine is able at the same time to keep up the initial blood pressure and maintain a normal glomerular function. That caffeine is *not* a decisive factor in this result is evident from the fact that ephetonin alone (Experiment 7) shows the same effect as ephetonin + caffeine (Experiments 5 and 6)..

In our opinion, the pronounced difference between Experiments 1 to 4 and Experiments 5 to 7 as far as the calculated amounts of filtrate are concerned may be explained only by inferring that it is due to the action of ephetonin on the circulation and, through this, on the blood pressure, an effect which, as far as we know, is entirely peripheral, independent of the central nervous system (16, 17, 18, 19).

Even though *we do not hold ourselves competent to enter into further pharmacological considerations on the action of ephetonin (and caffeine) —especially the action on the kidneys—we still think it justifiable to consider our findings as among that increasing number of experiments which supports the filtration reabsorption theory, inasmuch as our findings show consistently that administration of ephetonin or ephetonin + caffeine is able to prevent that fall of filtration which otherwise appears invariably coincident with the fall of blood pressure under spinal anesthesia.*

To us it does not seem possible to explain all the results of our experiments from the viewpoint of the secretion theory. For how would a substance like ephetonin, with a purely vascular effect which counteracts the fall of blood pressure that otherwise accompanies spinal anesthesia, be able also to compensate for a decrease of the kidney secretion due to that blocking of the secretory nerves which results from spinal conduction anesthesia?

SUMMARY

1. In 4 young men, cardiorenally healthy, the course of the glomerular filtration ("creatinine clearance," Rehberg) has been followed while the subjects were under spinal anesthesia, during which the fall of blood pressure was not compensated. The findings were:

a. During the rather considerable fall of blood pressure which appeared when the effect of the anesthesia was at its height, the glomerular filtration decreased markedly and to a degree that corresponded fairly well with the fall of blood pressure.

b. At the same time, the concentration index increased significantly and remained high, or rose even further during the subsequent gradual rise of blood pressure.

Thus the tubular function was normal, as far as the reabsorption of water is concerned, or even hypernormal at the same time that the effect of the spinal anesthesia was at its height and the filtration was lowered.

c. During the fall of blood pressure there was an enormous decrease in the urine volume, partly on account of considerable reabsorption of water in the tubules, partly on account of the lowered filtration in the glomeruli.

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THE MEASUREMENT OF THE INTRAPLEURAL PRESSURE IN MAN AND ITS SIGNIFICANCE

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The intrapleural pressure is generally regarded as merely an observation made during the institution of artificial pneumothorax. It is true that scattered observations on the intrapleural pressure in various pathological conditions have been made, but it does not seem to have been sufficiently realized that since the elastic properties of the lung have been subjected to a very careful analysis, this measurement has graduated from an empirical observation to one of real physiological significance.

Carson (1820) was the first to measure the elastic tension of the lung. This was done on the cadaver by connecting the trachea to a water manometer and observing the positive pressure developed when the pleural cavities were opened to the atmosphere. Todd, in 1849 (Heynsius, 1882), using the same technic, showed that the degree of redistension was proportional to the positive pressure applied intratracheally. These experiments were repeated in 1853 by Donders (Wirz, 1923), using a mercury manometer, a doubtful improvement in technic which does not seem to justify the term "Dondersche Druck," which is still used to describe the intrapleural pressure by certain investigators. These observations have been confirmed and repeated, with minor modifications, on lungs from most of the common pathological conditions (Perls (1869), Heynsius (1882), Liebermeister (1907), Romanoff (1911), v. Neergaard (1929) and Van Allen and Wu (1932)). The significance of such observations, if applied to the condition of the lungs in vivo, seems very doubtful, since Van der Brugh (1900) has clearly shown that death immediately results in a profound alteration of the elastic properties of the lung. We have observed the same phenomenon ourselves. The validity of measuring the elastic properties of a lung by inflation with positive pressure is also questionable, since Cloetta (1913) was unable to obtain any correspondence between such measurements and measurements made by inflation with negative pressure using a plethysmograph. A chronological review of the advances which have been made in our appreciation of the significance of the intrapleural pressure would be confusing. Instead, we have selected for description the forces which, according to present conceptions, are exerted on the lungs and will deal with these in turn.

The *intrapleural pressure* is the tension exerted on the surface of the lungs, either when at rest, as in apnoea, or when in motion, as when breathing. With the lungs at rest this pressure is termed the *static intrapleural pressure*, and in the simpler case, where the glottis is open and where, therefore, the intra-alveolar pressure is atmospheric, the elastic tension of the lung is exactly counterbalanced by the elasticity of the chest wall, the tonus of the respiratory

muscles and the weight of the abdominal viscera (Rohrer (1916 and 1925), Wirz (1923), etc.). Distribution of pressure throughout the intrapleural potential space is to all intents and purposes even (Rohrer (1916), Graham (1924), Wirz (1923), v. Neergaard and Wirz (1927)), which is what one would expect, considering the elastic properties of the lung (vide infra). Theoretically, a slightly more negative pressure might be expected at the apex than at the base, owing to the weight of the lungs themselves, but in the human subject this difference is too small to be demonstrable (Wirz (1923), Rohrer (1925)). At the hilus there may be a slight difference of pressure (Wirz (1923)) but only at the reflection of the visceral and parietal pleurae, where the structures that enter the lung differ in their elastic properties from the pulmonary parenchyma. A pressure gradient from hilus to periphery has not been established and indeed is not to be expected considering the almost perfect elasticity of the lung (vide infra).

In analysing the static intrapleural pressure with the glottis closed, the intra-alveolar pressure must be added to the elastic tension of the lung. The algebraic sum of these again is equal and opposite to the sum of the elasticity of the chest wall, the tonus of the respiratory muscles and the weight of the abdominal viscera. Changes in intra-alveolar pressure are conducted to the pleural surface (Cloetta (1913), Herbst (1932), etc.), with such rapidity that no lag can be demonstrated (v. Neergaard and Wirz (1927), Wirz (1923)). Some confusion exists as to the exact definition of static intrapleural pressure. This term is usually used to denote the intrapleural pressure at any degree of pulmonary distension, with no air entering or leaving the lungs and with atmospheric intra-alveolar pressure. The dynamic intrapleural pressure, on the other hand, is the intrapleural pressure taken at any moment during inspiration or expiration. Here the factors concerned are more complicated. The inertia of the moving viscera and air are negligible (Rohrer (1916 and 1925)). The resistance to the flow of air in the upper and lower air passages does influence however the intrapleural pressure through the medium of changes in the intra-alveolar pressure. It has been estimated (Rohrer (1925)) that approximately three-quarters of this resistance is located in the upper air passages and one-eighth in the bronchioles, and that, even during ordinary breathing, the intra-alveolar pressure fluctuates from approximately $+0.5$ cm. of water during expiration to -0.5 cm. of water during inspiration, while, during a test for vital capacity, the fluctuation may be 10 cm. of water, or more. The maximum and minimum intra-alveolar pressures during inspiration and expiration correspond to the maximum rate of flow of air and not to the degree of distension of the lungs. The changes in the calibre of the bronchial tree, which are known to occur during inspiration and expiration, make this correspondence only approximate (v. Neergaard and Wirz (1927)). In certain pathological conditions, such as bronchial asthma, the extent to which this fluctuation in the intra-alveolar pressure may modify the dynamic intrapleural pressure is of even greater significance (Hartwich (1930), v. Neergaard and Wirz (1927)).

The pulmonary elasticity has been subjected to a careful analysis and the evidence seems to indicate that, within the physiological limits of distention, the elasticity of the lung is nearly perfect.[1] It has been shown repeatedly that the

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[1] Considerable confusion exists as to the meaning of the term pulmonary elasticity. The standard definition of elasticity is the capability of recovering the original form on the removal of the load which causes deformation. The pulmonary elasticity is therefore the capability of the lung to recover its original size by elastic recoil, when that force tending to distend or compress it is re-

degree of distension is proportional to the tension exerted on the lung and vice versa (Heynsius (1882), Liebermeister (1907), Jaquet (1908), Romanoff (1911), Bernoulli (1911), Cloetta (1913), Leuret, Aumont and Delmas-Marsalet (1922), v. Neergaard and Wirz (1927), etc.). From the literature it can also be adjudged that, certainly within the limits of ordinary respiration and probably also within the limits experienced in measuring the vital capacity, the lungs can return to their original size by a process of passive elastic recoil. Within these physiological limits there is no demonstrable " set "; the elasticity of the lung must be very nearly perfect (Heynsius (1882), Liebermeister (1907), Jaquet (1908), Bernoulli (1911), Romanoff (1911) and Cloetta (1913)). When we consider that all uncontroversial evidence is in favour of expiration being a passive act (we are aware of the recent literature on electrobronchograms), it would be surprising if pulmonary elasticity under the conditions of the normal respiratory cycle were not perfect.

All are agreed that throughout the ordinary respiratory cycle the intrapleural pressure remains negative, both in animals and in man. The average values found at the end of inspiration and expiration by different investigators vary by about 2 to 4 cm. of water, but this may well be due to variations in the volume of the tidal air and the lack of standardization of technic, the importance of which is emphasized below. Without fear of contradiction, it can be said that the normal intrapleural pressure fluctuates around a mean of -5 cm. of water, or less, and that an ordinary breath is accomplished by a fluctuation in pressure of about 2 to 7 cm. of H_2O (Aron (1900 and 1928–29), Van der Brugh (1900), Rohrer (1925), etc.). The term pulmonary elasticity naturally focuses attention on the elastic fibres of the pulmonary parenchyma, but v. Neergaard has suggested that these may be less important than the surface tension of the alveolar walls. The nature of pulmonary elasticity has received but scant attention; it can only be suggested that the muscular and elastic fibres in the parenchyma, the surface tension of the alveolar walls and possibly the elasticity of the bronchi, the pulmonary vessels and the pleura are important factors.

The *pulmonary distensibility* is such that an ordinary breath results from a fluctuation in intrapleural pressure of from 2 to 7 cm. of water. It has been variously estimated, on the cadaver, on curarised animals, and by indirect methods of measurement on man, that pressure changes of from 4.5 to 10 cm. of water are required to bring about a volume change of one litre (Liebermeister (1907), Jaquet (1908), Bernoulli (1911), Cloetta (1913), Rohrer (1925) and v. Neergaard (1927)).

<div style="text-align:center">METHODS</div>

The technic of *measurement of the intrapleural pressure* has not undergone significant improvement during the past 80 years. Although many factors have been shown to influence intrapleural pressure. no attempt seems to have been made to control these by standardizing the conditions under which the measurements are made. We shall first deal with the technic of registering intrapleural pressure and then with those factors which may modify it.

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moved. The term *distensibility* is not a term used in physics but. rather than invoke Young's modulus of elasticity and surface tension. we have used it to define the ease with which the lung may be distended. By a decrease in distensibility we mean that a greater force has to be applied to the lung to distend it.

All methods employed involve the introduction of a small quantity of air into the pleural space and the transfer of the pressure fluctuation within this air space to some suitable recording system. It has been claimed by several investigators that the pressures so recorded do not represent the true intrapleural pressure, but reflect also the capillary pressure between the visceral and parietal pleurae. It has been shown that this artifact is however an insignificant factor, even with very small quantities of air in the pleural space (v. Stoevesandt (1911)). A more reasonable criticism is that the air injected collapses the lung and so changes the intrapleural pressure. This must always occur, but, in man at least, the effect of so minute a pneumothorax is small. From the numerous investigations which have been published on the relationship of pulmonary distension and collapse to intrapleural pressure (vide ante), we can assume that the change in pressure on the introduction of 40 cc. of air into the pleural cavity cannot possibly exceed 1 cm. of water [2] and in all probability is considerably less than ½ cm. of water. In those of our subjects in whom the true intrapleural pressure could be recorded after the introduction of 20 cc. of air, or less, the introduction of a further 20 cc. yielded no demonstrable change. It must be remembered that in animals whose lungs are small, the introduction of similar quantities of air may however be of profound significance.

To conduct the air pressure from this pocket of air in the pleural cavity to the recording system is a simple matter, but several technical points of prime importance must not be forgotten. Obviously the air passage between the lumen of the needle and the pleural space must be free. The free passage can be greatly facilitated by a side opening in the needle near the tip, but that it is adequate can only be established with certainty in our opinion, by a subsequent analysis of simultaneous tracings of intrapleural pressure and the volume of the tidal air (vide infra). The lumen of the conduit from the pleural space to the recording system must be of such dimensions that the resistance to air flow is not excessive. Although a bore of 3 mm. throughout the conduit system may be of advantage where time relationships are being studied (v. Neergaard and Wirz (1927), etc.), a one millimeter bore is ample for the measurement of the fluctuation in intrapleural pressure. We have used an 8.0 cm. trochar of 1.0 mm. bore and have conducted the pressure to our recording system by 100 cm. of tubing of 3.0 mm. bore. With a water manometer, this presents no disadvantages over 50 cm. of tubing of 5.0 mm. bore (Figure I, manometers A and B).

Considerable confusion evidently exists as to the relative merits of various methods of registering pressure. The use of tambours and optical registration is perhaps necessary for accurate measurement of the time re-

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[2] The pressures referred to are true pressures in centimeters of water. We cannot see any justification in measuring the change in only one arm of the manometer, as is the practice of some clinicians.

lationships between changes in intrapleural pressure and other physiological functions (v. Neergaard and Wirz (1927), Wirz (1923), Rohrer (1925)), but for quantitative measurements of the fluctuation of intrapleural pressure, the water manometer remains the instrument of choice. Optical registration is cumbersome and translation into a quantitative record of the changes in intrapleural pressure a matter of careful calibration. A water manometer undoubtedly has both lag and overswing but, if properly constructed and used, the errors accruing are negligible.

In Figure I, a recording manometer (A), as described by Masters (1930), is placed in parallel with a recording tambour, both being simultaneously subjected to a fluctuation of pressure from an artificial respiration
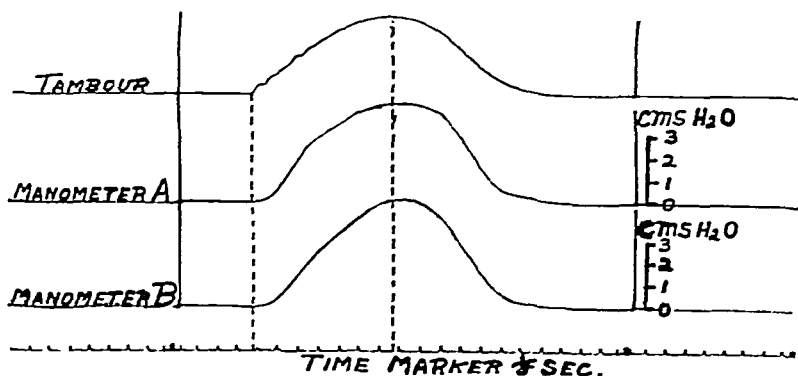
FIGURE I. CALIBRATION OF WATER MANOMETERS WITH TAMBOUR

Tambour and Manometer A connected to source of pressure fluctuation by 50 cm. tubing of 5 mm. bore. Manometer B by trochar and 100 cm. tubing of 3.0 mm. bore.

pump, arranged to deliver one cycle of 5 cm. of water pressure fluctuation in 2½ seconds. The phase relationship of the tambour and manometer tracings indicates that with the latter there is both lag and overswing. This is best shown by Manometer A (Figure I), where the tambour commences to rise 1/10 second before the water manometer. The subsequent rise of the tambour tracing shows no tendency to overfling, while the water manometer shows an inflection just before the rate of increase in pressure begins to diminish. When the pressure has reached its maximum a plateau is maintained and it is obvious that the maximum pressure recorded by the water manometer cannot be due to overswing, its natural period of oscillation being 0.23 second for a half cycle. The pressure changes recorded are greater and more rapid than those usually occurring within the pleura, and we are justified in assuming that the water manometer is well suited for recording static intrapleural pressure at the end of inspiration and expiration, although its use in recording dynamic intrapleural pressure

is quite unjustifiable. Manometer *B* (Figure I) shows the same pressure changes, conducted through the same trochar and tubing, as were used in the measurement of intrapleural pressures. The added resistance to flow of air which it offers increases the lag to $\frac{1}{4}$ of a second but damps down some of the overfling of Manometer *A*. Again this added resistance can in no way affect the maximum and minimum pressure readings.

The range of any sensitive water manometer of this type is limited to some 20 to 30 cm. of H_2O, which is insufficient to record the pressure change during the course of a measurement of vital capacity, or even of the tidal air in certain pathological conditions. To surmount this difficulty we have reduced the fluctuation of the recording manometer by inserting, in series, water manometers of exactly the same bore. Each manometer inserted in this way reduces the fluctuation of the recording manometer twofold. We have constructed a series of six such manometers, so equipped with taps that one or more can be switched in series with the recording manometer, yielding a range which extends from the fluctuation of a water manometer to that of a mercury manometer. A single many-way tap is used for each manometer. Although entirely satisfactory, such a complicated system is unnecessary for the registration of the intrapleural pressure during the course of a test for vital capacity. Only one or two manometers, in series with the recording manometer, are necessary for this purpose, and these may either be directly connected by rubber tubing, or, with a 3-way glass stopcock for each limb of the accessory manometers, one or two can be switched in series at will.

With an even distribution of pressure throughout the potential pleural space, it should not matter where the needle or trochar is inserted. This view has been generally accepted and, in most publications, the site of insertion is not even mentioned. Unfortunately, certain secondary factors make standardization of the site of injection of air into the pleural space essential. The most significant of these factors, and the one which has apparently not been appreciated, is the change in the *degree of distension* of the lungs with changes in posture. When the position of the subject is changed from recumbent to sitting, the intrapleural pressure, at the end of both inspiration and expiration, falls several centimeters of water. The change is accompanied by an increase in the amount of air in the lungs of several hundred cc. (Figure II). In repeating this manoeuver in various subjects we have found decreases in the intrapleural pressure of 3.2, 2.8, 2.0, 2.0, 2.4 cm. of H_2O, accompanied, in the last 4 cases, by increases in the functional residual air of 235, 230, 376 and 439 cc. respectively. Similarly, with elevation of the arms while in the recumbent position, decreases of 0.7 and 0.9 cm. in the intrapleural pressure were observed. It has long been known that changes in posture are associated with changes in the functional residual air (for references see Christie, 1932) and it is obvious that such a change must be accompanied by changes in the intrapleural
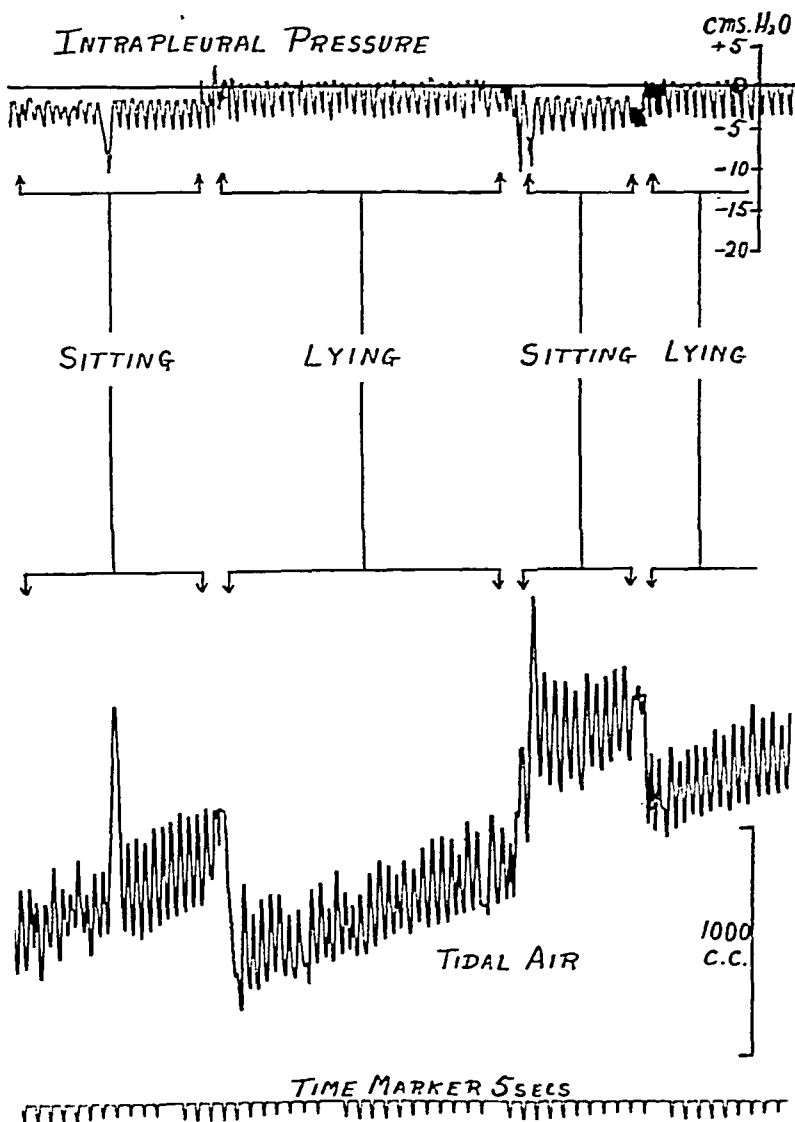
FIGURE II. THE INFLUENCE OF POSTURE ON THE INTRAPLEURAL PRESSURE AND THE RESPIRATORY LEVEL

Case 6. Minimal tuberculous lesion at left apex. Pneumothorax with 50 per cent collapse and no adhesions. On sitting up the rise in the respiratory level is accompanied by a fall in intrapleural pressure.

pressure. Changes in intrapleural pressure with posture have been described by Aron (1891) in a case of empyema. His observations were made in 1891 and, with the methods then at his disposal, it is hardly surprising that he did not suspect a change in the functional residual air or

realise the significance of his results. It is evident from our experiments that the various positions and the various approaches to the pleural space which are commonly employed may yield different intrapleural pressures. The mid-axillary line is perhaps most commonly used, either with the patient lying on his side or upright. With either of these positions the pressures recorded will differ from those obtained in the mid-clavicular line with the patient recumbent. The standard procedure that we have adopted presents many advantages. With the patient in the recumbent position, the arms by the side and the head supported by one pillow, a trochar such as described above is inserted into the 2nd or 3rd interspace in the mid-clavicular line. This site presents many advantages which have apparently never been realised: the air injected is localised around the needle point by gravity so that, the pneumothorax being minimal, a true pressure reading is secured; for this reason, and also since there are no blood vessels of great size in the vicinity, it is as safe as, if not safer than any other locality; with deep inspiration the excursion of the visceral pleura over the parietal is minimal and a tracing of the intrapleural pressure during a measurement of vital capacity is safe; the extrinsic muscles of respiration are relaxed so that some constancy of the resting respiratory level, and therefore of the intrapleural pressure fluctuation, may be expected; the patient is relaxed and can be expected to maintain this position for the duration of the experiment; most patients are comfortable in this position and, with the exception of those with extreme orthopnoea, all can tolerate it; lastly, and of considerable importance, it is possible in this position to obtain, simultaneously and without discomfort to the patient, a record of the tidal air as well as the intrapleural pressure.

Changes in the functional residual air with excitement and other external stimuli have also been described (Christie (1932)) and to reduce these to a minimum we previously accustom the subject to vital capacity exercises on a recording spirometer.

Changes in respiratory resistance as in bronchial asthma have been shown to produce a proportionate alteration in the dynamic intrapleural pressure through the medium of changes in intraalveolar pressure. Per se, however, they cannot influence the static intrapleural pressure at the end of inspiration or expiration, since, at this instant there is no flow of air to or from the lungs and the resistance is zero. If the resistance be extreme, the dynamic intrapleural pressure may actually be higher during expiration and lower during inspiration than the static intrapleural pressure at the end of expiration or inspiration. The fluctuations of intrapleural pressure here represent the extreme values of the dynamic rather than the static intrapleural pressure. This has been shown to be the case during an attack of bronchial asthma (v. Neergaard and Wirz (1927)), but does not occur with the small resistances such as are encountered with nasal breathing or breathing into a spirometer.

Occasionally, the excitement of creating an initial pneumothorax brings about an expiratory stridor or grunt, which may yield sufficient expiratory resistance to distort the pleural pressure tracing. The dynamic intrapleural pressure on expiration may then exceed the static yielding a characteristic tracing with a sharp peak during expiration and a subsequent fall to the static intrapleural pressure (Figure III). Once seen, this type of tracing can always be recognized, and the static pressure differentiated from the overswing. It is seldom encountered and is usually only a transitory phenomenon.

Indirectly, the intrapleural pressure may be altered by respiratory resistance through the medium of changes in the functional residual air (Christie (1932)). The small resistance which accrues from changing from mouth to nose breathing, or to breathing from a spirometer, is quite insufficient however to change the respiratory level (Greene (1933)). We have never observed a change in intrapleural pressure, nor indeed is such a change to be expected.

With the patient breathing into a recording spirometer, such as is used in the routine estimation of the basal metabolic rate, it is possible to obtain simultaneous records of the tidal air and the intrapleural pressure. The respiratory resistance in the spirometer circuit never exceeds ½ cm. of water, a resistance which we have found insufficient to produce a change in the maximum and minimum intrapleural pressures.

The classification of the lung volume and its subdivisions and the technic of measurement of the functional residual air have been described elsewhere (Christie (1932)).

<center>RESULTS</center>

It is surprising that the significance of simultaneous quantitative tracings of the tidal air and of intrapleural pressure does not appear to have been appreciated. Such tracings are obviously a record of the efficiency of the respiratory mechanism, the change in intrapleural pressure, with inspiration, representing the force exerted and the tidal air the work done. We have in other words, a complete record of the distensibility of the lungs in vivo and, in those pathological conditions when expiration must be an active rather than a passive act, of the compressibility of the lung. Furthermore, the elastic properties of the lung can be defined in considerable detail. We have not had the temerity to measure the intrapleural pressure on normal individuals but, since artificial pneumothorax is frequently made in the treatment even of minimal tuberculous lesions, there is ample material at hand to form an estimate of the tensile properties of what is, functionally at least, the normal lung.

<center>(a) Pulmonary distensibility</center>

We have defined a unit of pulmonary distensibility as the force, in centimeters of water, which must be applied to the surface of the lungs
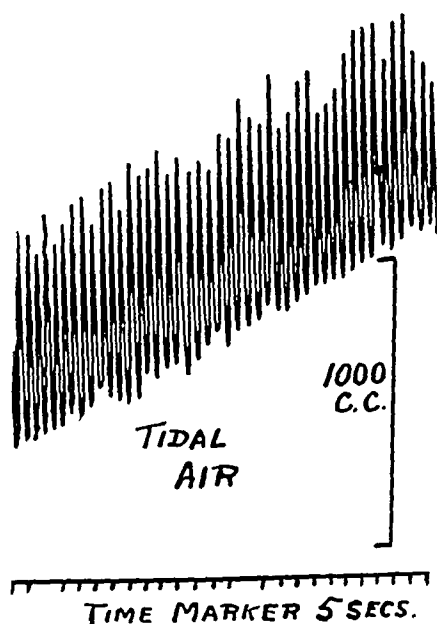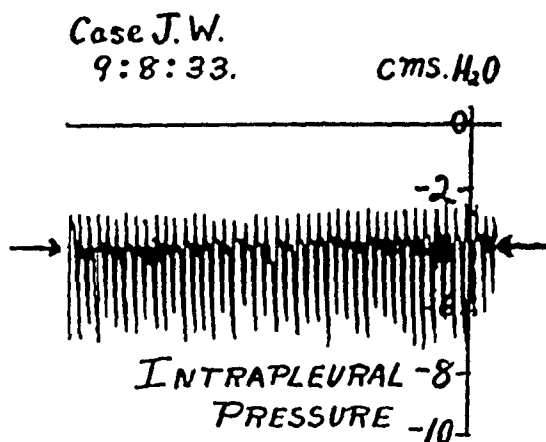
FIGURE III.   SIMULTANEOUS TRACING OF TIDAL AIR AND INTRAPLEURAL
PRESSURE

Case 5.   Initial right pneumothorax of 40 cc.   Minimal tuberculous lesion
at apex.   Tracing shows expiratory "overswing" due to "grunting" expira-
tion.   True static intrapleural pressure marked by arrow,

to yield an increase in lung volume of 100 cc. In a single individual this figure is remarkably constant and is independent of the depth of breathing (Figure IV). The stress is, in other words, proportional within physio-
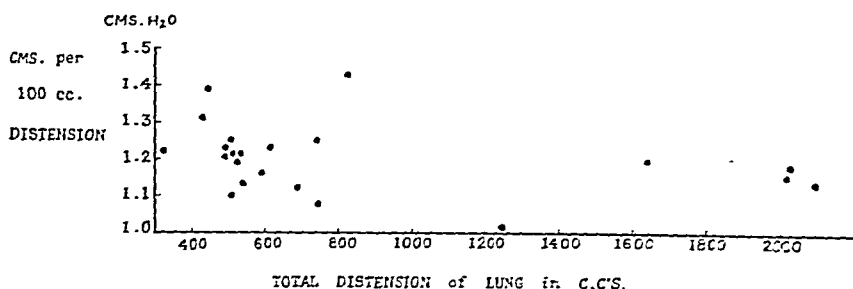
FIGURE IV. PULMONARY DISTENSIBILITY AND ELASTICITY
Case 3. Minimal tuberculous lesion at right apex. The depth of inspiration is plotted against

$$\frac{\text{Total change in intrapleural pressure}}{\text{Depth of breath in cc.}} \times 100.$$

Data obtained on two separate days, but always with a negative intrapleural pressure during both inspiration and expiration.

logical limits, to the strain, and the living lungs can be said to obey the laws of elasticity. From individual to individual we have found that the distensibility expressed in this way may show, however, wide variations (Table I). This must be expected, since the size of lungs varies. We have found that pulmonary distensibility may be profoundly impaired in several pathological conditions and it is obviously important to have a standard normal measure which will not vary with the stature of individuals. Since the depth of an ordinary breath is approximately 20 per cent of the functional residual air (Christie (1932)), we have adopted as the " coefficient of distensibility " the force, in centimeters of water, re-

TABLE I

Pulmonary distensibility

Case	Functional residual air	Distensibility in centimeters of H_2O per 100 cc. distension	Coefficient of distensibility *
	cc.		
1	1300	1.38	3.6
2	1400	1.98	5.5
3	1770	1.15	4.1
4	2030	1.43	5.8
5	3775	0.53	4.0

* The force in centimeters of H_2O required to distend the lung by 20 per cent of the functional residual air.

quired to distend the lungs by 20 per cent of the functional residual air. In 5 cases with minimal tuberculous lesions, this coefficient was found to range from 3.6 to 5.8 (Table I), a comparatively small variation when we consider that in cardiac failure it may rise to 20 or more.

(b) Pulmonary elasticity

The proportionality between the force exerted on the lungs and the degree of distension has already been emphasized and is in itself indicative of perfect elasticity. The number of observations required to establish such a proportionality is large (Figure IV), but there are less tedious methods of analysing the elastic properties of the lungs (Figures V and VI).

It is only with lungs of perfect elasticity that the points of maximum and minimum lung volume correspond to minimum and maximum intrapleural pressure. At the end of an ordinary or of a deep inspiration, the rate of volume change slowly diminishes until, at the height of inspiration, no air enters the lungs and there is consequently no further volume change. This is the point at which the static inspiratory intrapleural pressure is measured. It is obvious that, should there be impairment of pulmonary elasticity, this pressure cannot remain constant during " inspiratory apnoea," but will tend to return towards the pressure of the atmosphere. This is the course of events in emphysema, but, in normal lungs, the maximum degree of distension corresponds very closely to the point of most negative intrapleural pressure, even during a deep breath (Figure V).[3] This relationship also holds between the minimum degree of distension and the least negative intrapleural pressure.

The test may be carried further by instructing the subject to hold his breath with the glottis open, either in inspiration or expiration. If elasticity is unimpaired, intrapleural pressure remains constant and coarse oscillations corresponding to the heart beat are seen (Figure VI).

Simultaneous registration of tidal air and of intrapleural pressure yields a direct proof that true values of intrapleural pressure are being recorded. Since no two inspirations or expirations are identical either in volume or rate, the record of intrapleural pressure should reflect these differences and irregularities. If these are present, the record may be accepted as representing true fluctuations in static intrapleural pressure.

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[3] In Figure II we considered that it would be safe to investigate the influence of posture only on subjects with a moderate rather than a minimal pneumothorax. The subject from whom Figures V and VI were obtained also had a pneumothorax of moderate size and we have reproduced these, rather than those with a minimal pneumothorax, to facilitate comparison with emphysematous and cardiac subjects, where the mean intrapleural pressure is also atmospheric.
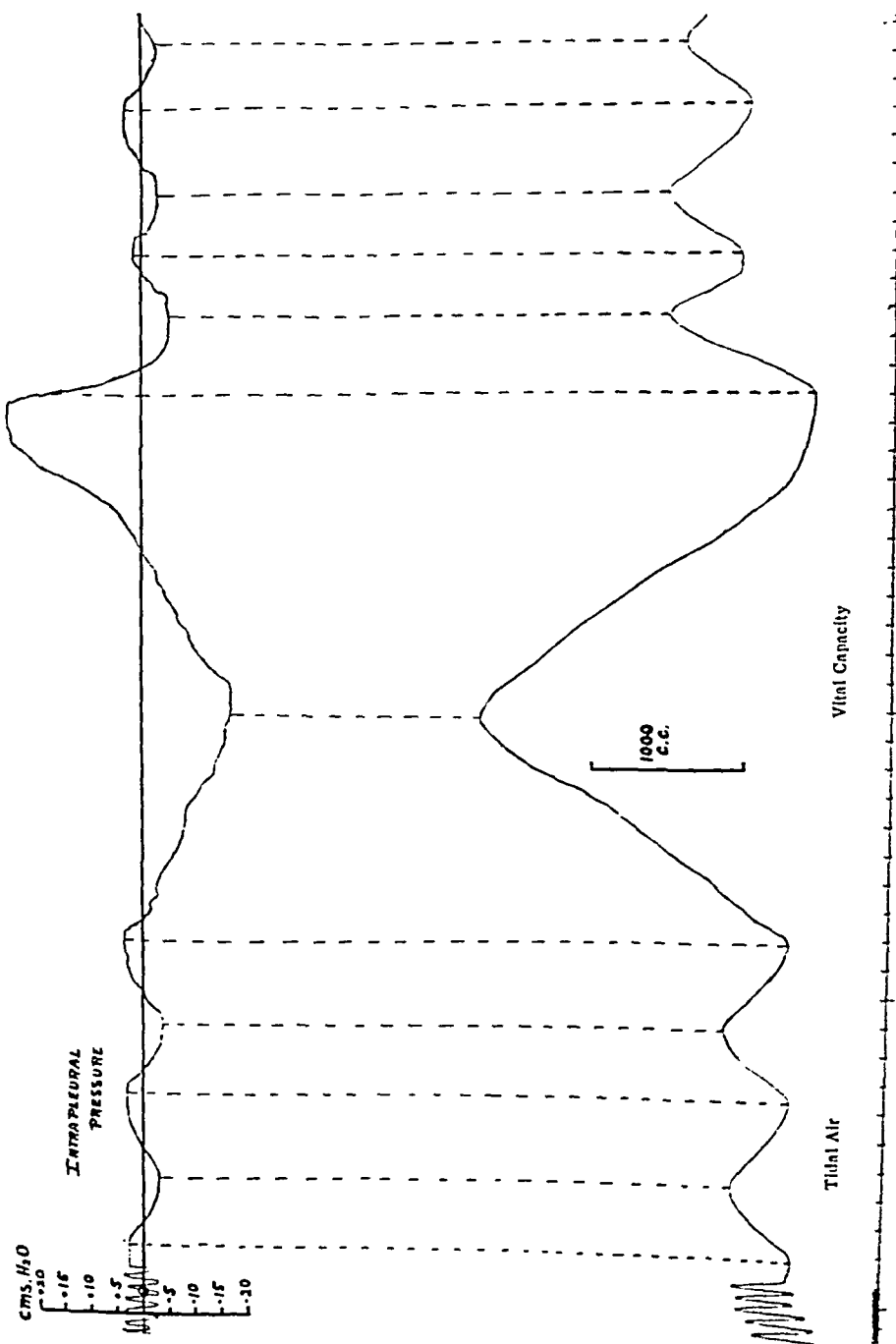
FIGURE V. PULMONARY DISTENSIBILITY AND ELASTICITY

Case 3. Minimal tuberculous lesion at right apex. Tracing to show that the point of maximum distension corresponds closely to the time when the intrapleural pressure is most negative.
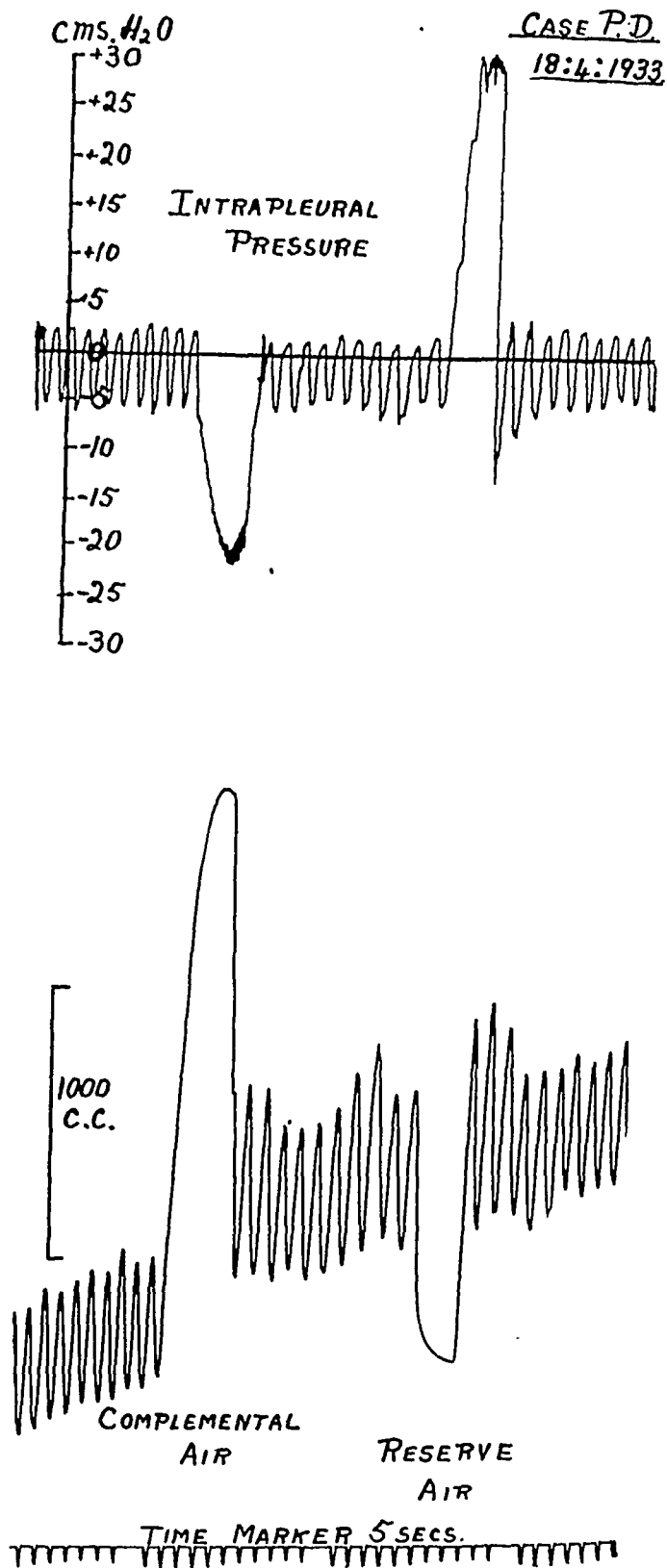
FIGURE VI. PULMONARY DISTENSIBILITY AND ELASTICITY

Case 3. Minimal tuberculous lesion at right apex. With either inspiratory or expiratory apnoea with the glottis open, the intrapleural pressure maintains its negative or positive pressure respectively. During this apnoea, coarse pressure fluctuations corresponding to the heart beat may be observed.

SUMMARY AND CONCLUSIONS

1. The significance of the static and dynamic intrapleural pressures and their relationship to pulmonary elasticity is discussed.

2. The methods which have been used for the measurement of intrapleural pressure are reviewed and evidence is given that, with the technic commonly employed, fortuitous variations, amounting to several centimeters of water, are to be expected.

3. A simple method for the simultaneous registration of intrapleural pressure and tidal air is described and the significance of such tracings, as a measure of the elasticity and distensibility of lungs in vivo, is discussed.

4. The elasticity of healthy lungs in vivo is shown to be perfect within the limits of experimental error, which are shown to be small.

5. The distensibility of healthy lungs, as measured by change in volume, with any given change in intrapleural pressure is shown to vary with the size of the lungs. A moderately satisfactory coefficient of distensibility is suggested which is independent of lung volume.

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THE ELASTIC PROPERTIES OF THE EMPHYSEMATOUS LUNG AND THEIR CLINICAL SIGNIFICANCE [1]

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In spite of much dogmatism in the literature, the etiology of pulmonary emphysema remains largely a matter for conjecture. It is clear that what is apparently true emphysema can be produced experimentally by several simple procedures, but their very simplicity and difference makes for scepticism in ascribing etiological significance to any one factor.

Respiratory resistance has long been suspected to be a factor in the production of emphysema. Although the etiological significance of glass blowing and the playing of wind instruments is not so certain as the text-books perhaps imply (Becker (1911), Tendeloo (1925), Jagić and Spengler (1924), Loeschcke (1928)), there is no doubt that experimental obstruction of the trachea or bronchi can occasion a lesion which is indistinguishable from the variety of emphysema which follows bronchial asthma (Sudsuki (1899), Nissen (1927) and Loeschcke (1928); Kountz, Alexander and Dowell (1929)). The anatomical changes are presumably due to overdistension of the alveoli, from the resistance to expiration, a factor probably of supreme importance in emphysema following bronchial asthma. Over the controversy whether there is atrophy of the elastic fibres or whether they are merely overstretched, it suffices to say that, in this type of emphysema, there is histological evidence of functional impairment of the elastic fibres of the lungs (Eppinger and Schauenstein (1904), Orsós (1907)), and that this impairment is secondary to overstretching of the alveoli. Similarly the loss of elasticity of the pleura and costal cartilages and immobilisation of the chest wall are probably secondary to increase in lung volume, and no clinical improvement follows mobilisation of the chest wall by surgical means (Hofbauer (1925), Nissen (1927)). There are several forms of emphysema which are, however, not preceded by bronchial asthma or any other demonstrable form of bronchial obstruction such as: so-called senile emphysema, emphysema of middle age, which is associated with kyphosis and other changes of the bony thorax (Loeschcke (1911), Tendeloo (1925), Loeschcke (1928), Kountz and Alexander (1933)). emphysema of those acclimatized to high altitudes (Campbell (1928 and 1929), Hofbauer (1925)), and patchy emphysema following heart failure with congestion, lobar pneumonia, pulmonary atelectasis, and pneumothorax. These conditions are not usually associated with obstruction to respiration. Although numerous theories have been advanced, it is difficult to draw any conclusion as to etiology from

the experimental production of emphysema by acclimatization to high altitudes, by removal of one lung, or by any procedure which lessens the amount of functioning pulmonary tissue. A priori it would seem reasonable to expect a "compensatory emphysema," either where a whole lung or portions of a lung are called upon to hyperventilate for a considerable period of time. This assumption does not bear careful analysis, and, in a subsequent communication, we will give evidence that the etiological factor in these forms of emphysema may well be circulatory rather than respiratory.

There may or may not be an etiological factor common to all types of emphysema but, both from a histological and functional point of view, certain features seem to be invariably present. Without going into controversial details as to the nature of the changes in the alveolar walls, it can be said that the walls are thin, stretched and torn, the respiratory bronchioles enlarged and distorted, and many of the capillaries torn and obliterated. The lungs do not collapse when the thorax is opened at autopsy and their appearance is one suggestive of impairment of their elastic properties. Considerable differences of opinion exist, however, as to whether there is a true loss of elasticity in emphysema. Measurements of the pulmonary elasticity at autopsy have yielded conflicting results in the hands of various observers, and few or no conclusions can be drawn from them (Tendeloo (1925), Loeschcke (1928), Thies (1932)). The significance of postmortem measurements of pulmonary elasticity has already been questioned (Christie and McIntosh (1934)).

During life there is evidence, although mostly indirect, that a loss of pulmonary elasticity exists in emphysema. From the somewhat dubious method of measuring the elastic recoil of the lungs from the intratracheal pressure recorded during complete obstruction to a passive expiration, Rohrer (1916) suggests that there is some such loss. The pleural pressure during respiration may fluctuate around that of the atmosphere (Kountz, Pearson and Koenig (1932), and the same changes have been shown to occur in experimental obstructive emphysema (Nissen and Cokkalis (1925–26), Kountz, Alexander and Dowell (1929)).[2] These changes would naturally follow loss of elasticity but we have been unable to find a quantitative measurement, or even proof of such a loss. The functional significance of impairment of pulmonary elasticity in emphysema seems, indeed, to have been ignored.

METHODS

The measurement of pulmonary elasticity and distensibility involves the simultaneous registration of the intrapleural pressure and of the volume of tidal air (Christie and McIntosh (1934)). The measurement of the lung volume and its subdivisions has also been described in a previous communication (Christie (1932)).

Samples of alveolar air were collected by the standard Haldane-Priestley procedure and also, in most cases, by the Henderson-Haggard automatic sampler (1925), modified in the following respects. The capacity of the sampling tube is reduced to 25 cc., so that if from 3 to 5 cc. of air be trapped at the end of each expiration, 20 breaths are sufficient to flush the tube with alveolar air. The

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[2] We would not include the cases of v. Neergaard and Wirz (1927), since from the protocols they were evidently asthmatic while the measurements were being made.

pressure fluctuation in the mouthpiece between the inspiratory and expiratory valves is conducted to a recording tambour so that any respiratory irregularities will be revealed. It is obvious that a single shallow breath results in a fallacious alveolar sample. The tambour tracing reveals such irregularities; it has been our custom to accept only those samples the average tidal air of which is over 400 cc. and when 20 regular breaths precede the removal of the sample. The *expired air* is collected in a Douglas bag while the sampler is operating so that, by dividing the volume of expired air by the number of respirations recorded by the tambour, the average *tidal air* can be calculated with considerable accuracy. The calculation of the *dead space* is rendered more accurate, or rather less inaccurate.

In patients who suffer an impairment of respiratory function, we have found the Haldane-Priestley method unsatisfactory and often obviously fallacious. In many cases, and this refers more especially to those of circulatory failure and those conditions such as artificial pneumothorax in which there is a reduction of the reserve air, this modification of the Henderson-Haggard automatic sampler is of definite use. In normal individuals this sampler yields results which are very slightly lower than those obtained by the Haldane-Priestley method (Table I).

TABLE I

The alveolar air of two normal subjects, measured by the automatic sampler and by the Haldane-Priestley method

Case	Number of observations	Automatic sampler pCO₂ Maximum	Minimum	Average	Average respiratory quotient	Number of observations	Haldane-Priestley pCO₂ Maximum	Minimum	Average	Average respiratory quotient
		$mm.$ Hg	$mm.$ Hg	$mm.$ Hg			$mm.$ Hg	$mm.$ Hg	$mm.$ Hg	
L.O.	16	41.7	37.7	39.1	0.79	22	42.9	36.4	39.7	0.76
W.D.P.	7	37.6	43.2	40.3	0.83	5	44.0	39.5	40.9	0.87

In many cases of circulatory failure and in cases of pneumothorax and thoracoplasty (Christie and McIntosh, unpublished data), in which the use of the Haldane-Priestley method is either inaccurate or impossible, this sampler yields results which correspond closely to the gas tension of the arterial blood. In emphysema neither method yields true samples of alveolar air and the use of the sampler is especially unsatisfactory on account of shallow breathing, but gives information on the gas pressure gradient between the alveolar and expired air.

The *arterial blood* is analysed for CO_2 and O_2 content and capacity by the method of Van Slyke (Peters and Van Slyke (1932)), each estimation being done in duplicate. The pH is measured by the colorimetric method of Cullen (Peters and Van Slyke (1932)), each estimation being checked with a sample of plasma of known CO_2 content and tension. The CO_2 tension is calculated from the CO_2 content and pH by the Henderson-Hasselbach equation.
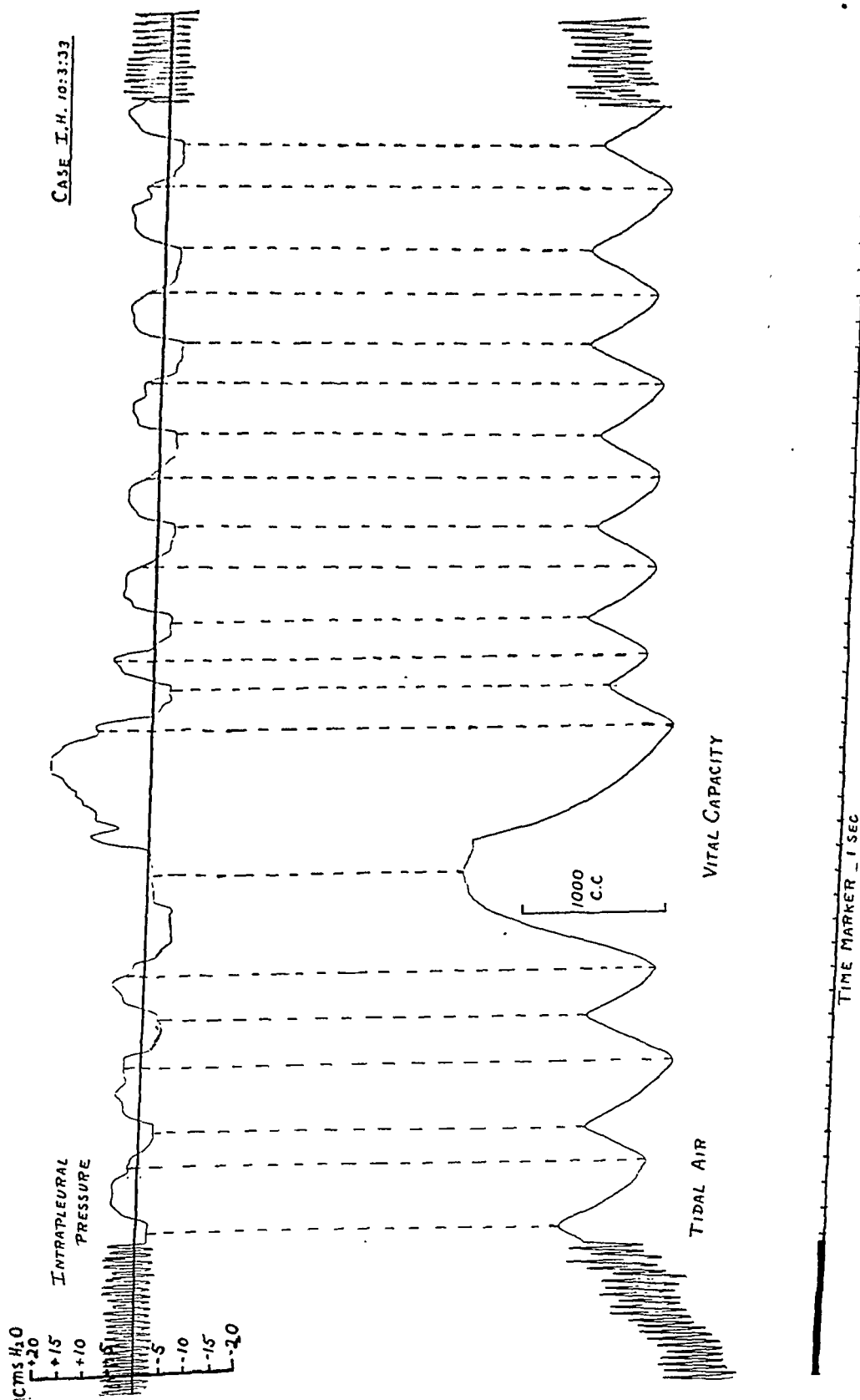
FIGURE II. THE INTRAPLEURAL PRESSURE IN EMPHYSEMA

Case I. H. Initial 40 cc. pneumothorax right side. Pressure fluctuates around that of the atmosphere. With deep inspiration the negative intrapleural pressure is held rather than increased, and when inspiration is complete pressure returns to atmospheric (To compare with normal see Christie and McIntosh (1934), Figure V).
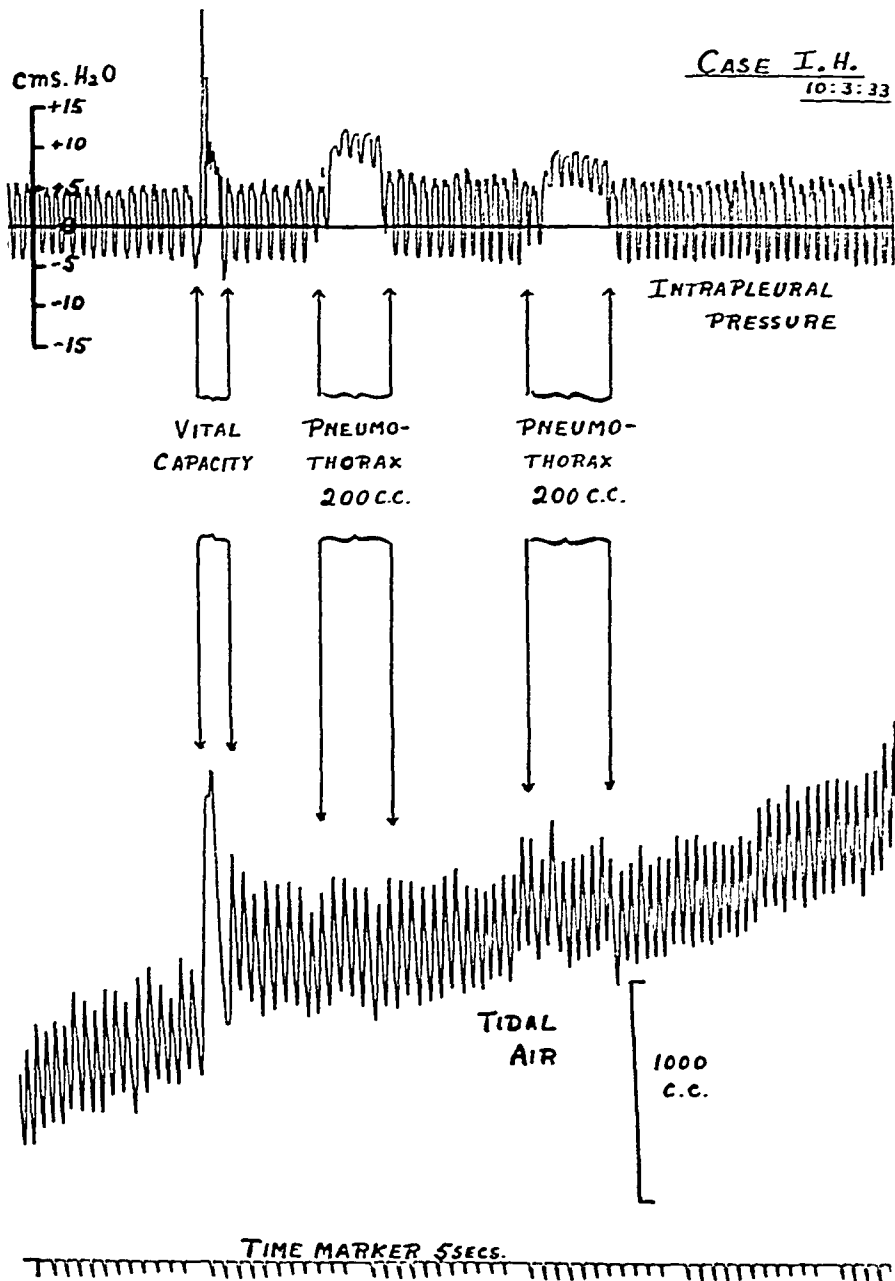
FIGURE III. PNEUMOTHORAX IN EMPHYSEMA

Case I. H. Tracing taken immediately after Figure II. A pneumothorax of 400 cc. does not change the intrapleural pressure in emphysema. (The rise registered while the air is being injected is due to the artifact of pressure resistance in the tubing.) (Reproduction of the normal will be published in a later communication on pneumothorax.)
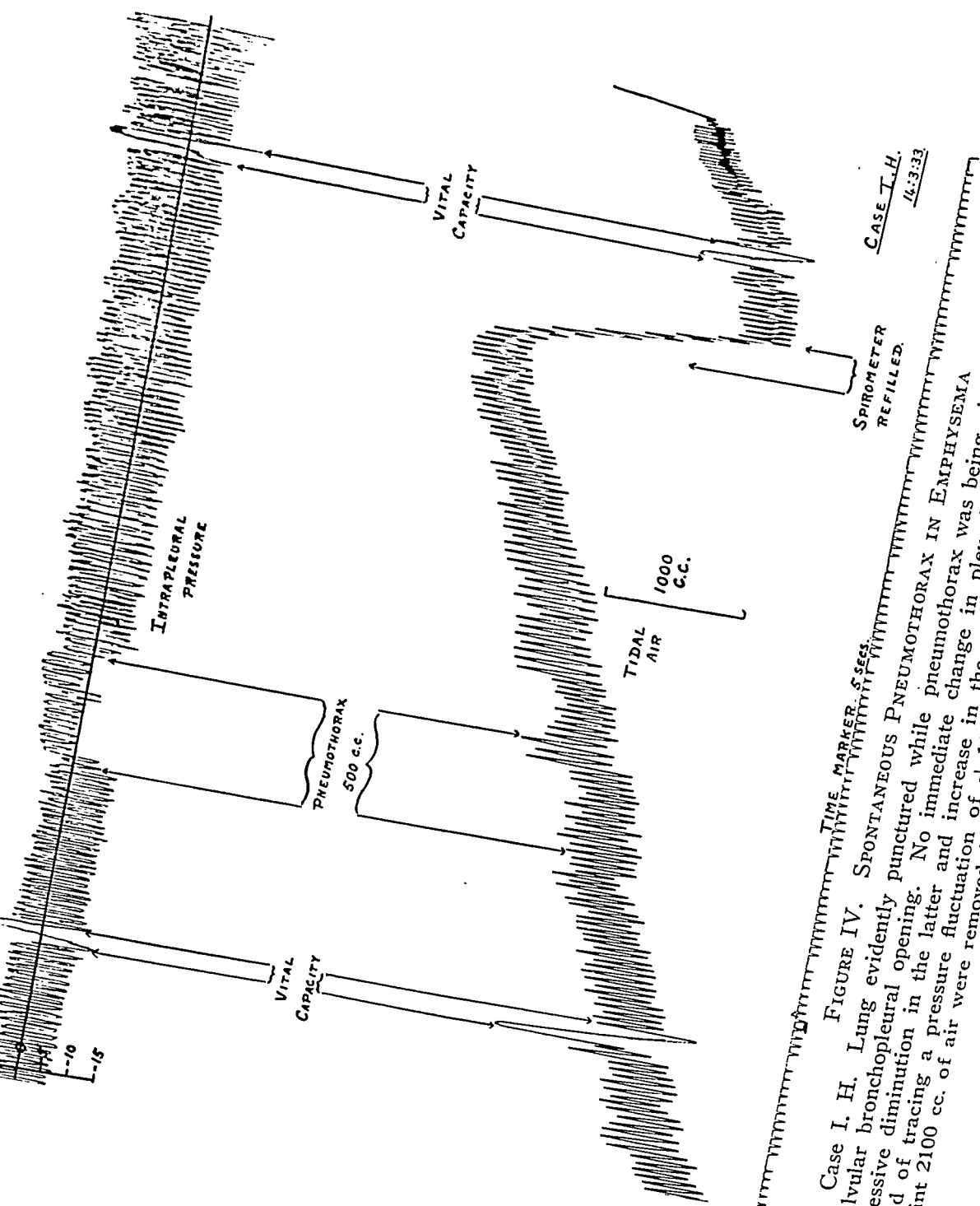
FIGURE IV. SPONTANEOUS PNEUMOTHORAX IN EMPHYSEMA

Case I. H. Lung evidently punctured while pneumothorax was being given with formation of valvular bronchopleural opening. No immediate change in the latter and increase in the expiratory pressure or tidal air but progressive diminution in the end of tracing a pressure fluctuation of $+13.2/-9.0$ only yielded a tidal air of 60 cc. At this point 2100 cc. of air were removed from the pleural cavity with complete relief of symptoms.

Pneumothorax has been advocated in the treatment of emphysema (Ganter (1926)) but, even with our limited experience of two cases, we believe it to be a dangerous procedure. This danger is well illustrated in Figure IV, a unique tracing of the onset of spontaneous valvular pneumothorax. The puncture evidently occurred while creating the pneumothorax, but we did not suspect an accident until some 5 minutes later, when the patient was requested to take a deep breath in order to measure the vital capacity. This manoeuver evidently exaggerated the leak into the pleural cavity, and the patient began to show definite signs of respiratory distress. One minute later a fluctuation in pleural pressure of $+ 13.2/- 9.0$ only yielded some 60 cc. of tidal air and profound anoxemia was evident. At this point 2,100 cc. of air were removed from the pleural cavity, with complete relief of symptoms. It was necessary to withdraw air continuously for the next 2 hours. Although the patient asked for another " treatment " the next day, we have discontinued pneumothorax therapy in emphysema. We suspect that a similar, but less severe leak, occurred in Case S. H. and we know of at least one unpublished accident of this sort in another clinic. Aside from being a unique record of the occurrence of spontaneous pneumothorax, Figure IV also illustrates the loss of pulmonary elasticity in .emphysema. A pneumothorax of 500 cc. had no effect on intrapleural pressure (the rise while the pneumothorax is being given is an artifact due to inflow of air), and, as more air leaked into the pleural cavity, the decrease in tidal air was not accompanied by a change in negative intrapleural pressure. At the very end of the tracing, with the patient in extremis and gasping for breath, there is a change, but this is in all probability due to an effect on the dynamic, rather than on static, intrapleural pressure.

We would like to emphasize that pneumothorax therapy has been advocated in the treatment of emphysema (Ganter (1926)), and that these measurements of intrapleural pressure were made in the course of an attempt to assay this method of treatment. Case S. H. felt definitely worse after a pneumothorax of only 200 cc. but an x-ray showed 20 per cent collapse of the lung so that we suspect that a small spontaneous pneumothorax occurred. Case I. H. was treated with artificial pneumothorax on 3 occasions and after each claimed slight symptomatic improvement, although objectively no change was noticed. The high incidence of spontaneous pneumothorax can only be ascribed to the weakness of the visceral pleura in emphysema or to the rupture of a pleural bleb and. in our opinion, contra-indicates the use of this form of therapy.

II. The vital capacity

Fortunately it is possible to estimate the loss of pulmonary elasticity in emphysema without measuring intrapleural pressure, by the simple procedure of taking a vital capacity tracing on a recording spirometer. If a

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normal individual be instructed to take a deep breath, he will subsequently expire all, or almost all of the air inspired, returning to his previous respiratory level. In other words, there is no demonstrable " set " when the lungs are distended by a deep inspiration. In most early, and all advanced, cases of emphysema the lungs are overstretched when the subject inspires deeply, with a consequent failure of the next expiration to return to its previous respiratory level. A new level may be adopted, the lungs being more distended than before, but usually the respirations " step down " until a constant level is reached in the space of 12 breaths or more. The same phenomenon may be observed on deep expiration; this can only be due to overstretching of lungs the elasticity of which is impaired. This overstretching or overdistension can be made more obvious if the patient be told to expel a sample of reserve air immediately after the forced inspiration. Since the lungs have been overdistended, this sample of reserve air is much smaller than one taken after a period of quiet respiration. A slight discrepancy between the reserve airs taken in these two ways is only occasionally observed in normal individuals, whereas in emphysema a marked difference is the rule rather than the exception.

These characteristics of emphysematous lungs are well shown in Figures V and VI, Cases A. N. and F. F. being moderate and L. L. and M. S. advanced cases of emphysema (also S. H. in Figure II of Christie, 1932). In Table II we give a synopsis of the measurements of vital capacity in all our cases of emphysema. No cases were discarded in which a diagnosis of emphysema was made and of which tracings of the vital capacity were taken. In Cases I. H., S. H., and L. L., the reserve air taken in these two ways always showed this characteristic discrepancy, while in cases S. G., and M. S., the reserve air was a negative quantity after a deep breath. Cases C. N. and F. F. only sometimes showed overdistension but in an average of several observations it could be shown to exist. Hurtado, Fray and McCann (1933) [3] have confirmed these observations in only 2 of 9 cases of pulmonary emphysema. Some of these cases, however, were not previously accustomed to vital capacity exercises, and only 2 of 9 had a reduction of the vital capacity to less than 2,700 cc. None of our cases had a vital capacity of over 2,600 cc. (Table II), and in those in which it exceeded 1,600 cc. a positive " overdistension test " could be demonstrated only by averaging several observations. The significance of this overdistension test in cases of mild or moderate emphysema is uncertain and remains to be proved, but its value is evident in advanced emphysema both as a gauge of loss of pulmonary elasticity and in differential diagnosis from reduction of vital capacity due to congestive heart failure. The simplicity of this " overdistension test " enhances its value as a diagnostic sign. Very

[3] Dr. Hurtado has asked me to correct a misprint in this article. The legend to Figure 2 should read " Black dots are cases of pneumoconiosis; circles are cases of pulmonary emphysema."
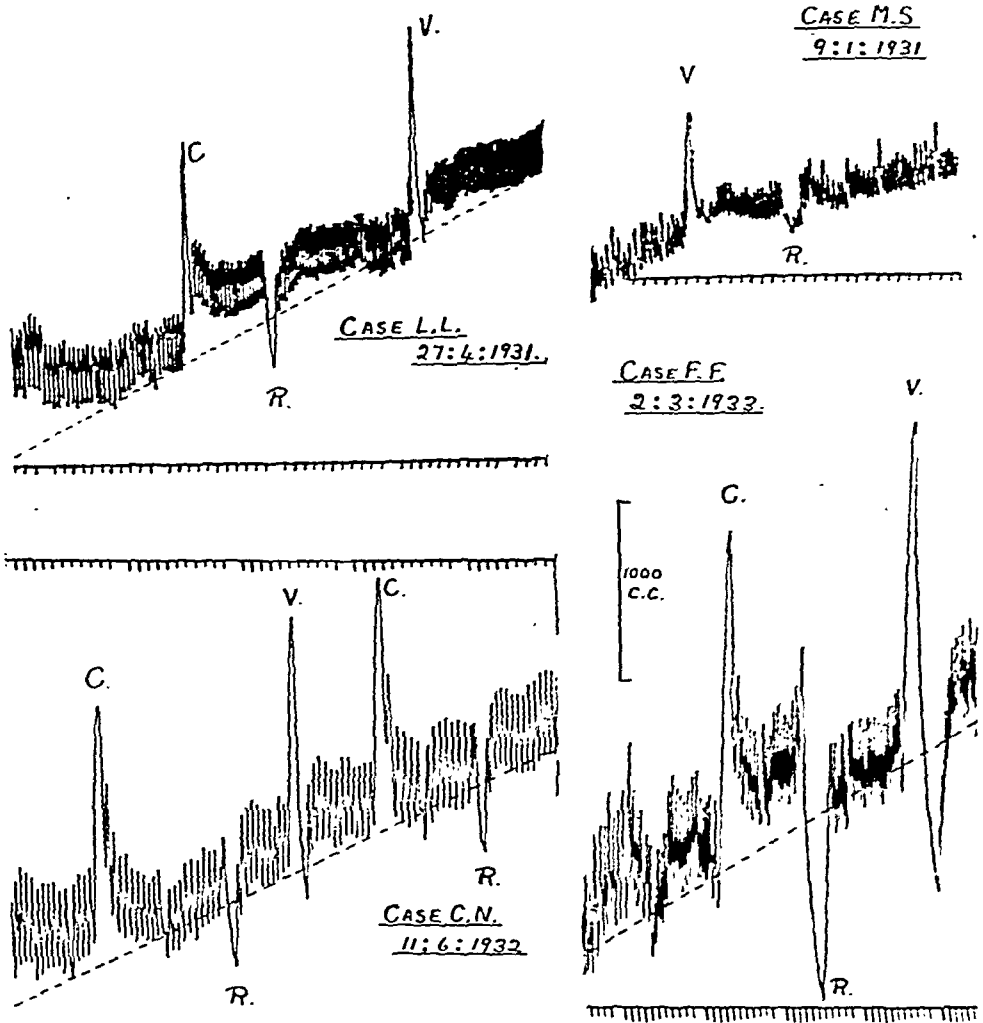
FIGURE V. THE VITAL CAPACITY IN EMPHYSEMA

The vital capacity in 4 cases of emphysema showing the overstretching on deep inspiration and the difference between the volume of reserve air taken alone and taken at the end of a test for vital capacity.

C = Complemental air
R = Reserve air
V = Vital capacity.

The irregularity in the resting respiratory level is also well shown.
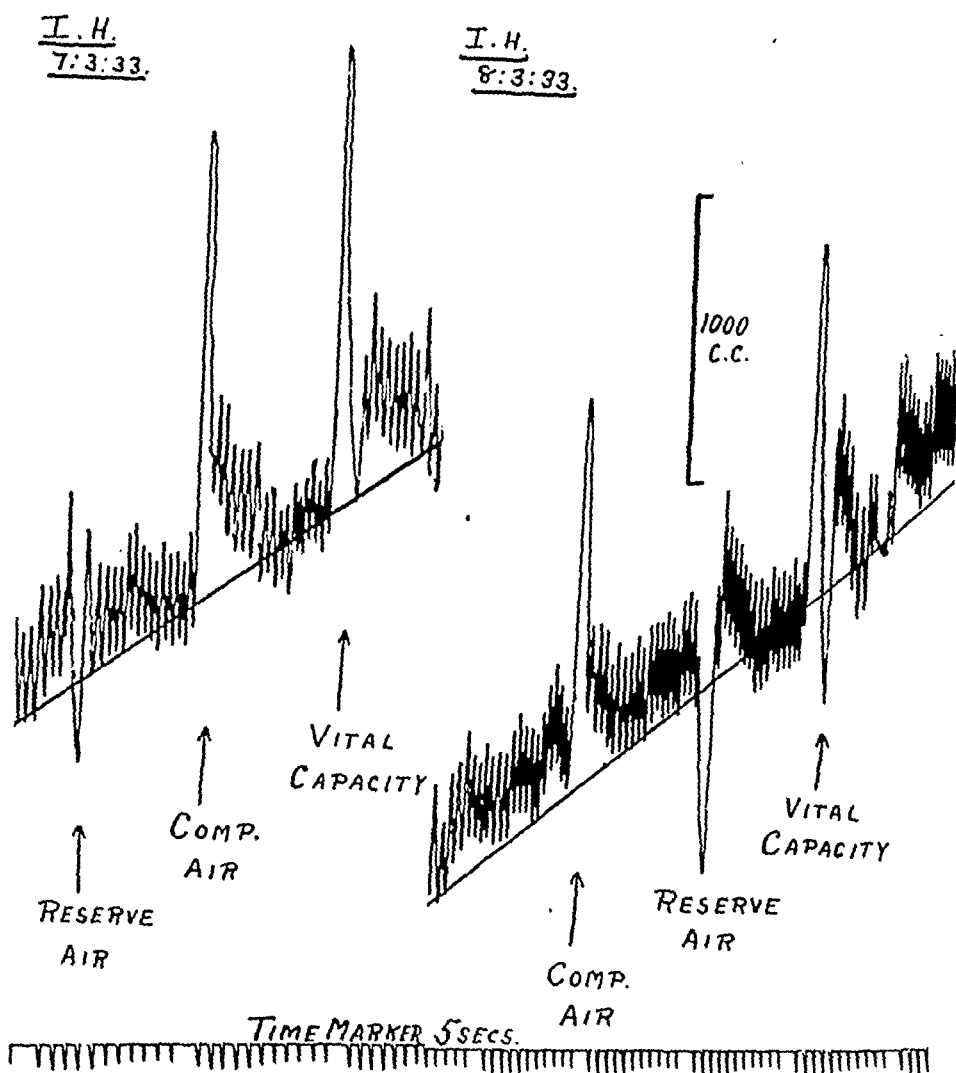
FIGURE VI. THE VITAL CAPACITY IN EMPHYSEMA

Illustrating the same points as Figure V, and also the changes in the respiratory level which can be observed from day to day.

TABLE II

The vital capacity in emphysema

Case	Reserve air		Complemental air	Number of observations
	After deep inspiration	After normal inspiration		
	cc.	cc.	cc.	
I.H..............	97	352	1432	10
S.H..............	—83	261	878	2
C.N..............	268	335	1375	3
L.L..............	—60	230	1254	2
F.F..............	784	985	1792	6
S.G..............	—66	(not recorded)	1104	3
M.S..............	—105	(not recorded)	680	5

little co-operation is required; it can be facilitated by using the technic we have already described (Christie (1932)) and by training the subject to measurement of the vital capacity.

III. *The tidal air*

The tidal air also shows certain characteristic features in emphysema.

The resting respiratory level fluctuates in a manner which must be considered abnormal. These fluctuations occur from minute to minute (Figures V and VI), rendering an estimate of the oxygen consumption very difficult, and also from day to day, with a corresponding fluctuation of the complemental, reserve and functional residual air (Figure VI). This irregularity in the respiratory level, associated with a loss of pulmonary elasticity, is interesting. The reflex control of the resting respiratory level has not yet been defined and any correlation with pulmonary elasticity can only be suggested.

IV. *The lung volume and its subdivisions*

The lung volume and its subdivisions also show striking abnormalities, which can be fully explained on the basis of a loss of pulmonary elasticity. These subdivisions have been analysed in 6 patients (Figure VII) but unfortunately it is impossible to form even an approximate estimate of what is normal in any individual, from his stature. A very constant relationship exists, however, among these subdivisions themselves (Meakins and Christie (1929), Hurtado and Boller (1933), etc.). Using exactly the same technic as ourselves for the measurement of the lung volume and its subdivisions, Hurtado and Boller (1933) have very carefully defined the limits of variation in 50 normal adult male persons, the vital capacity, functional residual air (mid capacity) and residual air averaging 78, 38 and 22 per cent of the total capacity respectively. (In 50 normal adult female individuals the corresponding percentages were 71.6, 44.7, and 28.3 (Personal communication).) In emphysema the ratio of functional residual air to total capacity is perhaps the most instructive. Instead of averaging 38 per cent, as in normal male persons, it ranges between 70 and 79 per cent (Figure VIII). In other words, the emphysematous subject breathes at a level which is only from 20 to 30 per cent below full chest expansion. This is indeed a remarkable deviation from the normal, when we consider that it represents a functional change rather than one which is purely anatomical. With the loss of elasticity, and consequent fluctuation in intrapleural pressure around that of the atmosphere, such an increase of the respiratory level is to be expected. In the normal individual the chest wall is continuously exerting traction on the lungs, but this force is counterbalanced by the elastic pull of the pulmonary elasticity. With loss of elasticity the lungs must yield to the traction of the chest wall and distend until
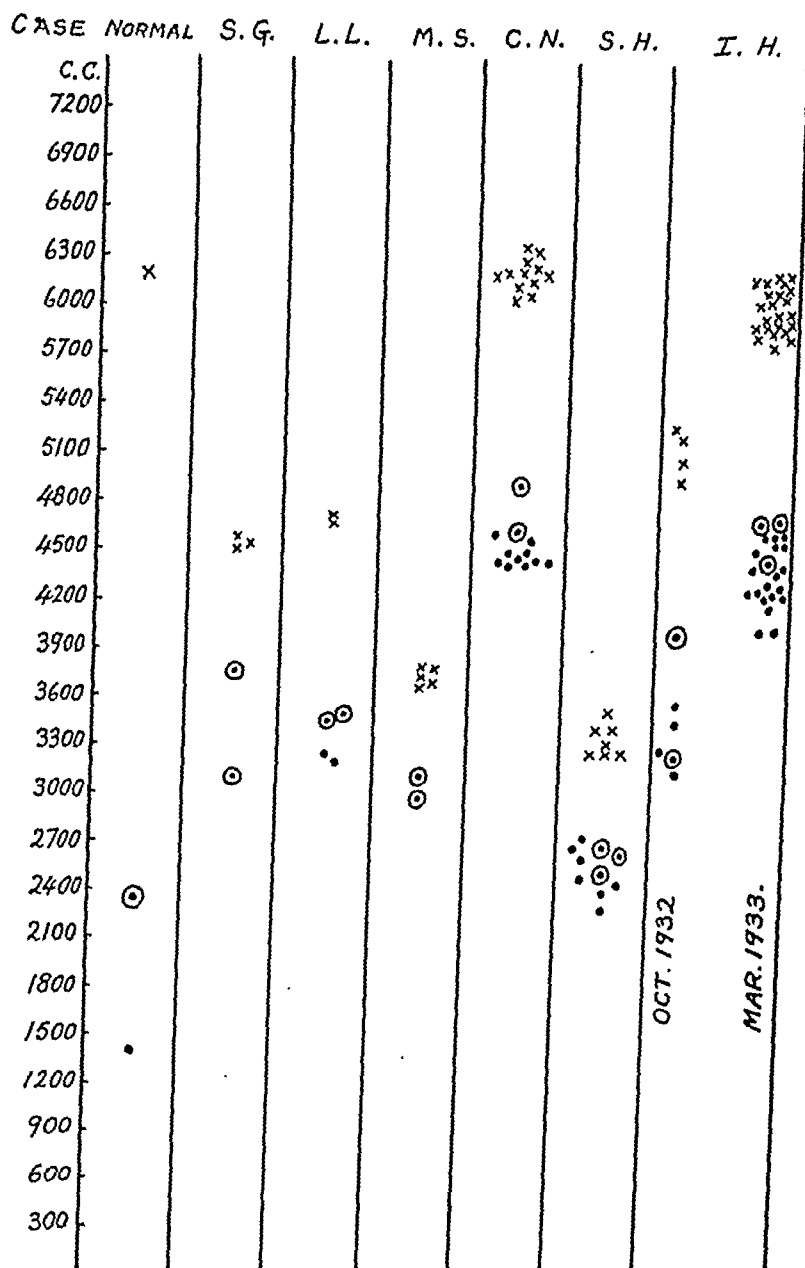
FIGURE VII. THE LUNG VOLUME AND ITS SUBDIVISIONS IN EMPHYSEMA

$X =$ Total capacity
$\odot =$ Functional residual air
$\cdot =$ Residual air.

this force is obliterated. In other words, the volume of functional residual air must increase until the intrapleural pressure fluctuates around that of the atmosphere. This increase in the volume of functional residual air must be at the expense of the complemental air, since it is the size of the thoracic cage which limits the volume of a forced inspiration. On the basis of loss of pulmonary elasticity we have then a reasonable explanation both
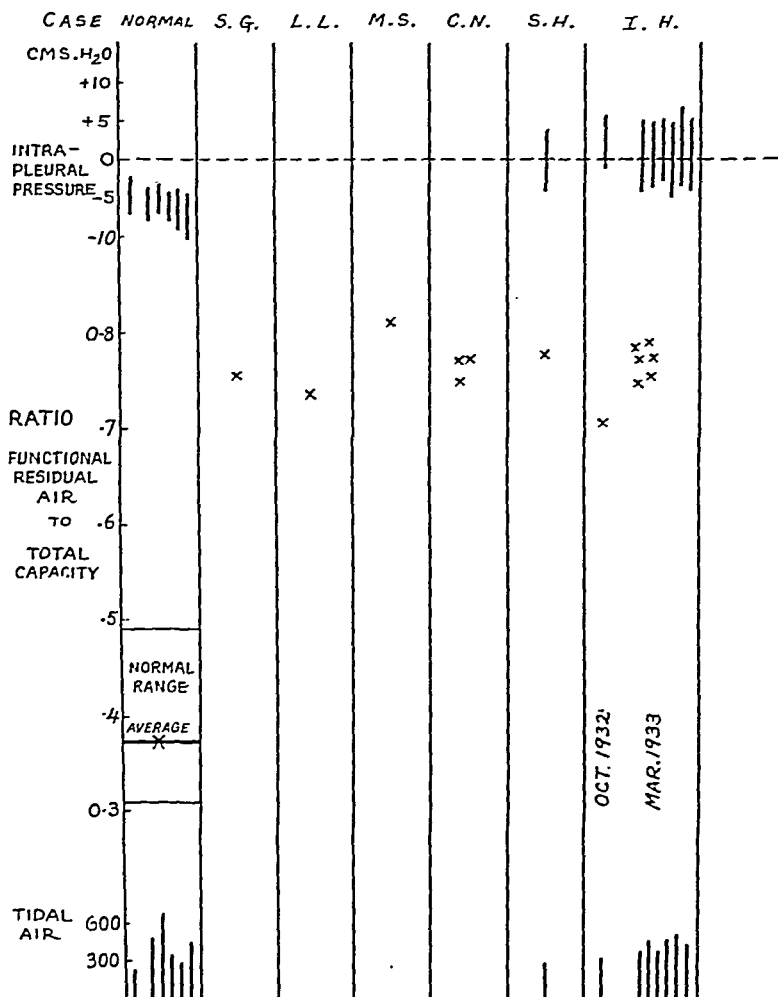
FIGURE VIII. THE RATIO OF FUNCTIONAL RESIDUAL AIR TO TOTAL CAPACITY IN EMPHYSEMA

$$X = \text{ratio} \frac{\text{Functional residual air}}{\text{Total capacity}}.$$

The average intrapleural pressure fluctuation with the corresponding tidal air are shown of Cases S. H. and I. H., and 5 normal individuals with functional residual airs ranging from 1,300 to 3,775 cc.

for increase in the volume of functional residual air and decrease in the volume of complemental air in emphysema. Another constant feature is the diminution, or even disappearance, of the reserve air. We believe this to be due indirectly to the positive intrapleural pressure which has to be developed for an expiration of any depth, another sequel of loss of pulmonary elasticity and one which will be discussed later.

Siebeck (1910), Bruns (1910), Bittorf and Forschbach (1910), Plesch (1913), Lundsgaard and Schierbeck (1923), Anthony (1930), and Herms and Rüttgers (1931) have also observed an increase of the volume of residual air in emphysema. We have repeated their observations since, with the exception of the two cases of Anthony (1930), the methods used were open to criticism (Christie (1932)). In a recent publication Hurtado, Fray and McCann (1933), using the same technic as ourselves, have observed the same tendency to a relative increase in the volume of functional residual air and of residual air and decrease in the volume of the vital capacity. The decrease in vital capacity was not so marked in most of their cases as in our own but it is interesting that in their Cases 2, 3 and 9, where the reduction of the vital capacity was comparable to that of our own cases, the ratio of the volume of functional residual air to that of the total capacity was also comparable i.e., between 70 and 80 per cent. In cases with a larger vital capacity the increase in this ratio was not so great.

V. Haemo-respiratory exchange

Details of haemo-respiratory exchange in our cases of emphysema are given in Table III. With the shallow breathing and diminution of the

TABLE III

Haemo-respiratory exchange in emphysema

Case	Tidal air	Respiratory rate per minute	O₂ consumption per minute	Alveolar pCO₂ (Haldane Priestley)	Alveolar pCO₂ (automatic sampler)	Expired pCO₂	Arterial blood O₂ capacity	O₂ saturation	pCO₂
	cc.		cc.	mm. Hg	mm. Hg	mm. Hg	volumes per cent	per cent	mm. Hg
S.G.	241	32.0	316.5	49.5	47.5	24.3		Marked cyanosis	
L.L.	276	27.0	261.0	40.8	35.9	19.6		Slight cyanosis	
M.S.	240	19.5	281.0	53.8	52.5	31.8		Marked cyanosis	
	227	24.8	312.0	58.1	50.6	29.0		Marked cyanosis	
	243	25.5	302.0	47.6	48.6	27.1		Marked cyanosis	
C.N.	379	27.3	303.7	47.8	36.4	18.1		No visible cyanosis	
	331	25.3	224.5	46.6	31.2	16.9		No visible cyanosis	
S.H.	183	36.5	214.8	68.4		21.8	26.34	77.0	83.0
	165	37.3	237.8	71.0	49.8	21.6	28.63	73.9	73.0
I.H. October 1932	265	19.3	201.8	39.3	33.7	16.6	19.39	90.1	47.0
March 1933	282	17.7	195.0	38.5	34.6	19.5	20.97	92.3	43.8
March 1933	311.5	15.7	190.5	45.4	37.2	21.0			

reserve air in all our cases, any direct measurement of the alveolar air must be of doubtful significance, the estimated value of the alveolar pCO_2 being probably always too low. Even with this error, the alveolar pCO_2 is high in all cases except L. L. and I. H. In I. H. the arterial blood indicates slight anoxemia and CO_2 retention, and L. L. exhibited slight but definite cyanosis. All our cases showed some evidence of impairment of haemo-respiratory exchange, most marked in that of S. H. and least marked in L. L. All showed a tendency to rapid and shallow breathing but this is most marked in those with the greatest impairment of haemo-respiratory exchange.

<div align="center">DISCUSSION</div>

I. The breathing in emphysema

The analysis of the elastic properties of the emphysematous lung has shown that there is an almost complete loss of elasticity, which in turn leads to increase of the functional residual air as the lung yields to the traction of the chest wall. The intrapleural pressure, fluctuates perforce around that of the atmosphere, so that expiration has to be performed by active muscular effort. The normal lung deflates by a process of elastic recoil, but in emphysema it must be compressed by positive intrathoracic pressure. The structure of the thoracic cage in man presents several drawbacks to this unnatural type of expiration. With the possible exception of the internal intercostal and anterior scalene muscles,[4] positive intrapleural pressure can be generated only by the extrinsic muscles of respiration. The diaphragm is a muscle of inspiration and, in the normal individual, is elevated during expiration by the elastic recoil of the lung. In emphysema elastic recoil is not only abolished but positive intrathoracic pressure is generated, which further impedes complete relaxation of the diaphragm during expiration. The inspiratory efficiency, or " stroke." of the diaphragm is consequently diminished. Diminution in the excursion of the diaphragm is a characteristic of emphysema and we believe that our conception of its cause is of fundamental importance in the symptomatic relief of the dyspnoea, which is so frequently observed in this condition.

To compensate for incomplete ascent of the diaphragm during expiration, the emphysematous subject may make an effort to increase the intra-abdominal pressure by contracting the abdominal muscles. Unfortunately, the abdomen is usually pendulous and the tone of the abdominal muscles poor, but the expiratory abdominal effort can usually be demonstrated by observing the abdominal relaxation and protrusion which in emphysema occur at the beginning of, and not during, inspiration (Staehelin and

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[4] The force exerted by these muscles must in any case be negligible but it would be interesting to observe at autopsy whether hypertrophy can be demonstrated.

Schütze (1912)). On a theoretical basis, anything which increases intra-abdominal pressure should tend to increase the efficiency of the diaphragm. The simplest method of accomplishing this is by means of an abdominal binder. We have tried this in two cases of emphysema with encouraging results. Symptomatic improvement is difficult to gauge objectively but both patients quite definitely state that with the abdominal binder they are more comfortable at rest and experience less dyspnoea on moderate exertion. One has been wearing a belt since November, 1932. An abdominal binder is not an impressive form of therapy to a patient complaining of shortness of breath and it seems unlikely that the improvement was due to suggestion. It is of interest that the patients know exactly the adjustment which yields most relief. With the belt either looser or tighter they realize that the optimum pressure is not being exerted. Reich (Ganter, 1926) observed the same clinical improvement and an increased excursion of the diaphragm in emphysema after pneumoperitoneum. This may well have been due to an increase in intra-abdominal pressure, similar to what we obtain with a binder. Alexander and Kountz (1933) have recently recommended for the symptomatic relief of emphysema a similar type of abdominal belt. Their explanation of the rationale of this form of treatment differs from our own, but in a comparatively large series of cases they have been able to note a high percentage of symptomatic relief. We cannot discuss their very interesting communication in greater detail, as it has not yet been published. Their results are certainly in perfect agreement with our own.

The positive intrapleural pressure developed during expiration has other clinical applications. The diminution, or even disappearance, of the reserve air, may be due to paradoxical movements of the diaphragm when the positive intrathoracic pressure is still further increased by an attempt at forced expiration. The paradoxical movements of the soft structures in the supraclavicular hollow and lower intercostal spaces, so often observed in emphysema, can also be explained on the basis of a positive intrathoracic pressure during expiration.

Prolongation of expiration is a text-book sign of emphysema and, if the respiratory curve be carefully analysed, presents irregularities which are strongly suggestive of active muscular expiratory effort, such as occurs in exercise (Rohrer (1916), Scott (1920) and Hartwich (1930)). Our intrapleural pressure tracings show this to be the case, since the generation of a positive intrapleural pressure can only be the result of an active muscular effort.

Rapid and shallow breathing is frequently observed in cases of advanced emphysema (vide infra). The peculiarities in the distensibility of the emphysematous lung, and the increased amount of work required to yield a breath of normal size, have already been emphasized. The relationship between pulmonary distensibility and the Hering Breuer reflex

will be analysed in a subsequent communication but, if we accept a tension change in the pulmonary parenchyma as underlying this reflex, it seems reasonable to suppose that the decrease in distensibility might be reflexly responsible for the rapid and shallow breathing frequently observed in emphysema.

It is possible that the irregularity of the respiratory level, which is characteristic of emphysema, may also represent a disturbance of the Hering Breuer reflex. With the loss of elasticity, changes in volume do not necessarily correspond with changes in tension. If a tension change be responsible for the Hering Breuer reflex, it is obvious that an irregularity of both inspiratory and expiratory respiratory levels is to be expected.

It has been shown that the emphysematous subject does not respond to the inhalation of carbon dioxide by the normal increase in ventilation (Scott (1920), Meakins and Davies (1925)), and an increased tolerance to CO_2 has therefore been suggested. Cases of emphysema with impairment of haemo-respiratory exchange may be acclimatized to a higher pressure of CO_2 than the normal, but they are actually unable to hyperventilate. For example, Case L. L., while under basal metabolic conditions, was requested to hyperventilate voluntarily to the limit of his capacity. The period of hyperventilation lasted for 20 seconds, but he was only able to raise his minute respiratory volume from 7.44 to 12.93 liters, and this although he had previously been accustomed to vital capacity exercises and was obviously over-exerting himself. The respiratory rate remained unchanged and the respiratory quotient rose from 0.80 to 0.915, which would certainly not indicate any effective hyperventilation. Hurtado, Fray and McCann (1933) have recently demonstrated in a severe case of emphysema inability to increase pulmonary ventilation on exercise. This inability to hyperventilate is not surprising. (a) The diminution in the efficiency of the diaphragm and (b) the paradoxical movements of the soft parts of the thoracic cage have already been explained. (c) The chest is already in the inspiratory position, with a consequent diminution of complemental air and inability to produce any significant increase in inspiratory depth. (d) Expiratory depth also cannot be increased owing to diminution, or even obliteration, of the reserve air. (c) Increase of depth is the result of increase in the duration of application of force, rather than increase in the magnitude of the force acting on the lungs. To breathe deeper the patient must breathe slower; or per contra, increase in rate tends to prevent an increase in depth. The last factor is probably the most important in the prevention of hyperventilation in emphysema.

II.   The circulation in emphysema

The positive intrapleural pressure which exists during most of the respiratory cycle must be reflected by increase in the venous pressure. This consequence has been analysed by Kountz, Pearson and Koenig (1932),

and will be discussed later in greater detail (Christie and Meakins (1934)). The absence of orthopnoea in true emphysema is noteworthy, when we consider that this symptom has been repeatedly ascribed to increase in venous pressure.

III. The distribution of the lesion in emphysema

It is obvious that, with loss of elasticity, a pressure gradient with inspiration and expiration will exist in the lung. The superficial alveoli will be subjected to the greatest stress and strain and will consequently be the first to overstretch. This distribution is characteristic of the lesion and its progression in emphysema.

IV. The impairment of haemo-respiratory exchange

The mechanism of anoxemia and CO_2 retention in emphysema can hardly be said to be even controversial, since there is little or no concrete evidence to support any of the various theories which have been submitted.

a. An impairment of diffusion between the alveoli and capillaries has often been suggested. Since the solubility of CO_2 is some 30 times that of O_2 such an impairment, if it did exist, would affect CO_2 rather than O_2 exchange. This is not the case.

b. Hypoventilation has also been suggested as a cause of anoxemia in emphysema (Houssay and Berconsky (1932)). It is true that some cases of emphysema do hypoventilate but hyperventilation is more frequently observed (Meakins and Davies (1925), Scott (1920), Staehelin and Schütze (1912), Hoover (1912), and Table III). Nor is there a direct relationship between anoxemia and rapid and shallow breathing, although the latter does usually occur in advanced cases.

c. A rather ingenious explanation has been suggested by Beitzke (1925), on the basis of imperfect mixing of gases in the alveoli. If a stream of air enter a cavity from a small opening, the air currents are such that the cavity is well ventilated. If the opening be large, the same volume of air entering the cavity may, however, produce very imperfect ventilation. In the same way he argues that ventilation of the normal alveolus is facilitated by the narrow opening of the alveolar duct into the atrium, and that in emphysema distension of this opening is responsible for an imperfect mixing of gases in the alveoli. His arguments are based on simple and large artificial models not wholly analogous to the alveolar ducts and spaces. From the laws of diffusion of gases, it can be calculated that such an impairment of ventilation could not be responsible for any significant damming back of CO_2 within the atrial system. If we take the model of a flat surface of 10 square meters from which 200 cc. of CO_2 are excreted per minute into perfectly still air, it can be calculated that if the pressure of CO_2 at a distance of 1 cm. from this flat surface is 40 mm.,

the pressure at the surface itself cannot exceed 40.2 mm. In other words, diffusion alone will prevent any significant damming back of CO_2 in the alveoli so long as *contact* is maintained between the inspired air and the air in the atrium. Beitzke does not doubt that inspired air. enters the atrium, but merely questions the ventilation of the alveoli. Since the openings of the alveoli into the atrium are wide, diffusion alone could not permit of any significant gradient of CO_2 between atrium and alveolus. It might be said that a surface molecular layer of CO_2 exists on the surface of the alveolar wall. Such layers are known not to obey the laws of diffusion, and an actual current of air blown over the surface of the alveolar wall might be essential to prevent their formation. But, during a slow, prolonged expiration, there can be no ventilation of the alveolar wall, and yet, once the dead space has been flushed through, the pressure of CO_2 in the expired air. shows a steady rise which corresponds to excretion of CO_2 into the alveoli (Haldane (1922)). This can only mean that during expiration, when there is no ventilation of the alveoli, CO_2 is transferred from the blood to the alveolus, and from there to the atrium and the expired air. The same steady rise in alveolar CO_2, corresponding to the rate of CO_2 excretion, can be demonstrated during voluntary apnoea. This seems to be excellent evidence against the presence of a significant molecular layer, and against the theory of Beitzke. It also supports our contention that ventilation of the alveolus itself is not a necessary part of the respiratory mechanism.

d. Hoover (1915) has suggested that " the real difficulty of ventilation in emphysema and asthma lies in the distension of the infundibula and this fails to allow an equal diffusion of carbon dioxide through the alveolar air." The rapidity with which CO_2 diffuses has already been emphasized and we cannot believe that this mechanism can be responsible for the impairment of haemo-respiratory exchange in emphysema.

e. Another theory, our own, may be described as follows. The elasticity of the healthy lung has been shown to be very nearly perfect. With pulmonary expansion the pressure change is consequently distributed evenly throughout. The even distribution of the expanding force is obviously essential for the uniform expansion of the lung and must be largely responsible for the normal equal distribution of ventilation throughout the alveoli. With a loss of elasticity, this expanding force must act mainly on the periphery of the lung, to be damped down as it passes to the hilus. The simile of a rubber elastic sponge will perhaps make this more clear. If a rubber sponge of perfect elasticity be compressed, the pressure is evenly distributed, and all the air spaces diminish in size. With complete loss of elasticity the same pressure compresses only the air spaces at the surface, which remain collapsed until a force is applied to distend them. We have demonstrated the perfect elasticity in the normal, and lack of elasticity in the emphysematous lung. and believe that this difference in the distribution

of distension exists in emphysema. Such inequality of distension should be reflected by unequal mixing of gases in the alveoli. There is ample evidence that this occurs (Bruns (1910), Siebeck (1911), Weiss (1928), Hoover (1915)). One criticism may be levelled against this theory of unequal expansion. From the dissociation curves of oxygen and carbon dioxide, such an inequality of ventilation should impair absorption of oxygen to a greater extent than excretion of CO_2, since hyperventilation of any area can blow off an excess of CO_2, but cannot overaerate the blood with O_2. There is no evidence, however, that excess excretion of CO_2 occurs. But if we assume a circulatory deficiency in the peripheral over-distended and overventilated alveoli, increased excretion of CO_2 in these areas would be much reduced and might well be offset by diminished excretion of CO_2 in the deeper under-ventilated alveoli, where ventilation depends partly on diffusion (CO_2 diffuses only 32/46 as rapidly as O_2).

The overdistended atria and alveoli are located for the most part in the peripheral portions of the lung, and present ample evidence of such an impairment of circulation. The capillaries are sparse and collapsed and frequently have ruptured. Ventilation of these peripheral portions of the lung must be largely wasted effort since they contain a much diminished proportion of the pulmonary circulation. Yet these areas must actually be hyperventilated at the expense of the more normal alveoli, situated deeper in the lung. Such a change in the distribution of ventilation should be reflected by a decrease in the effective tidal air, since some of the air inspired is shunted into ineffective alveoli deficient in blood supply. Calculation of the effective tidal air from the data available in the literature would indicate that its diminution is a characteristic feature of emphysema (Hoover (1912), Scott (1920), Meakins and Davies (1925) and Table III). From a functional point of view, the shunting of air into relatively ischaemic alveoli might appear to constitute an increase in the anatomical dead space. There are certain fundamental differences, however, which can serve to differentiate the two. With each breath the true dead space is flushed with alveolar air, whereas an ischaemic alveolus is merely a cul-de-sac, never flushed with either alveolar or room air. With an increase in the anatomical dead space, a larger expiration is required to flush it with alveolar air, with a consequent diminution in the rate at which the pressure of CO_2 will rise in the first portion of air expired. In emphysema the reverse can be shown to be the case: If expiration takes place through a long tube into a Douglas bag and samples of air be collected at intervals along this tube, by means of the automatic sampler described above, it can be shown that, in advanced emphysema, the pressure of CO_2 rises much more rapidly than normally. For example, in Case M. S., the pressure of CO_2 rose to 41 mm. of Hg, after only 100 cc. of air had been expired, whereas, normally a pressure of only 22 mm. of Hg, or less, is reached after 200 cc. have been expired (Christie and Loomis (1932)). These

figures are certainly not suggestive of any true increase in either the anatomical or physiological dead spaces in emphysema. The histological picture suggests some dilatation of the smaller bronchioles. But even if the volume of the bronchial tree is doubled, and if the volume of the tidal air is 300 cc., the dead space would not be more than 180 cc. It should be possible, moreover, to obtain a true sample of alveolar air during forced expiration of the tidal air plus 300 cc. reserve air. These are extreme values and yet, it is seldom if ever possible to obtain a sample of alveolar air in emphysema by this method.

On the basis of the histological and elastic changes passage of tidal air into the peripheral ischaemic and over-distended alveoli should occur. The natural functional sequelae should be diminution in effective alveolar ventilation and unequal mixing of gases in the lung. Both these functional abnormalities are invariably found and are not due to a simple increase in the respiratory dead space. This diminution in effective ventilation is of fundamental significance when we consider the drawbacks under which the respiratory muscles are working. At the beginning of inspiration they are already in the inspiratory position; a large proportion of the air inspired is wasted on functionless alveoli; and to top all, the lungs cannot passively relax in expiration except as the result of an unnatural expiratory effort. The whole cycle occurs so quickly that the component parts are not distinguished; the net result is just shortness of breath, increased by exercise and not relieved by posture, unless complicated by circulatory failure.

SUMMARY

1. From simultaneous tracings of the volume of tidal air and of intrapleural pressure in emphysema, an almost complete loss of pulmonary elasticity can be demonstrated.

2. The loss of elasticity can be more simply, but not so accurately, demonstrated by changes in tracings of the vital capacity. These are suggested as a crude clinical test for the loss of pulmonary elasticity in emphysema.

3. Characteristic changes in the lung volume and its subdivisions are described and an explanation for these changes advanced, on the basis of loss of pulmonary elasticity.

4. An analysis of haemo-respiratory exchange does not yield an explanation of the functional impairment in emphysema.

5. Evidence is presented, based on loss of pulmonary elasticity, that the peripheral, distended and relatively ischaemic alveoli are over-ventilated at the expense of the deeper and more normal alveoli. The subsequent diminution of the effective tidal air is held responsible for some, if not all, of the anoxemia and CO_2 retention observed in emphysema.

6. Expiration in emphysema is shown to be the result of a positive intrapleural pressure, generated by active muscular effort. The functional

drawbacks of this type of expiration are emphasized, in particular diminution in the excursion of the diaphragm, with consequent impairment of inspiratory efficiency.

7. It is shown that the emphysematous subject is incapable of effective hyperventilation, although hyperventilation is called for by the diminution in the volume of effective tidal air.

8. An increase of the intra-abdominal pressure, by means of an abdominal belt, is of value in the symptomatic treatment of emphysema. The rationale of this form of treatment is described.

9. A description of the functional impairment in emphysema may be condensed into the following brief summary. The muscles of inspiration are already in the inspiratory position when inspiration commences. A greater amount of work is required to distend the lung, and a large proportion of the air which is inspired is wasted on the peripheral functionless alveoli. Even after this wasted effort the lungs cannot passively relax except as the result of an unnatural expiratory effort. As respiratory compensation fails, imperfect aeration of the blood occurs, and a vicious circle is established, with increasing demand for hyperventilation and decreasing ability to ventilate.

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<div align="center">APPENDIX</div>

Case histories

Case I. I. H.—Male—Aet. 56.

Chronic cough for 10 to 15 years with dyspnoea on exertion progressively getting worse for the past 6 years. Recently has developed occasional oedema of ankles. Typical physical signs of advanced emphysema.

Heart from right sternal border to 10.5 cm. to the left of midline. Blood pressure 128/80. Liver and spleen not palpable. No oedema. No clubbing of fingers. Cyanosis questionable.

Diagnosis: Chronic bronchitis with emphysema.

Case II. S. H.—Male—Aet. 52.

Chronic cough for 30 years. Dyspnoea on exertion for 6 years. Orthopnoea and blueness of face for 3 years. Typical signs of emphysema with paradoxical movement of lower intercostal and supraclavicular spaces. No apparent enlargement of thoracic cage. Minimal bronchiectatic lesion at either base confirmed by lipiodol. Moderate kyphosis. No enlargement of heart. Liver edge palpable. Slight oedema of both ankles. Marked engorgement of retinal veins. Blood pressure 106/58.

Diagnosis: Chronic bronchitis, bronchiectasis and emphysema.

Case III. C. N.—Male—Aet. 43.

Bronchial asthma of 4 years duration. Dyspnoea on exertion of 5 months duration. Physical signs of advanced emphysema. Heart from right sternal border to 10 cm. to left of midline. Blood pressure 130/80. Liver and spleen not palpable. No oedema.

Diagnosis: Bronchial asthma and emphysema.

Case IV. M. S.—Female—Aet. 52.

Shortness of breath and swelling of abdomen and ankles—6 months' duration. Chest barrel shaped with typical signs of emphysema. Marked cyanosis. Heart 2½ cm. to right, 12 cm. to left. Blood pressure 134/84.

Diagnosis: Emphysema and cardiac decompensation.

Case V. L. L.—Male—Aet. 52.

Cough and shortness of breath—duration 12 years but progressively getting worse. Barrel shaped chest with classical signs of emphysema. No cardiac enlargement. Blood pressure 120/70.

Diagnosis: Chronic bronchitis and emphysema.

Case VI. F. F.—Male—Aet. 55.

Asthmatic bronchitis of 11 years duration. Recently has had some dyspnoea on exertion. No cyanosis. Definite signs of emphysema.

Diagnosis: Chronic bronchitis and emphysema.

Case VII. S. G.—Male—Aet. 44.

Asthma and bronchitis, 15 years duration. Breathlessness on exertion and blueness of face. Marked plum coloured cyanosis with all classical signs of advanced emphysema.

Diagnosis: Asthma, chronic bronchitis and emphysema.

THE INTRAPLEURAL PRESSURE IN CONGESTIVE HEART FAILURE AND ITS CLINICAL SIGNIFICANCE [1]

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The mechanism of cardiac dyspnoea remains a matter for conjecture although many hypotheses have been advanced. Most of these resolve themselves into a statement that a correlation between dyspnoea and some other physical sign exists. Reduction in the volume of the vital capacity, increase in the venous pressure, reduction in the cardiac output, and so on, have been suggested as the "cause" of cardiac dyspnoea, since they are usually proportional to the degree of respiratory distress. Most, if not all, of those hypotheses which blame impairment of one or other of the mechanisms which are known to control breathing, do not bear the test of experimental analysis. We will review in brief the various theories which have been advanced to describe the cause of cardiac dyspnoea.

Cardiac dyspnoea can conveniently be divided into four groups which are quite distinct clinically, and which presumably are caused by different mechanisms. These are orthopnoea, paroxysmal cardiac dyspnoea (cardiac asthma), Cheyne-Stokes respiration, and lastly dyspnoea on exertion, which, as heart failure with congestion progresses, becomes continuous dyspnoea exaggerated by exertion. The last type is a cardinal symptom of heart failure and the various theories which have been advanced as to its causation will be dealt with first.

a. Imperfect aeration of the respiratory centre, due either to diminished circulation rate of flow or to deficient aeration of the arterial blood, has long been emphasized as one of the primary causes of *cardiac dyspnoea*. Yet there is seldom any significant oxygen unsaturation of the arterial blood and the pressure of CO_2 is almost invariably below normal. The circulation rate is diminished in heart failure with congestion but the evidence is against any significant increase in the tension of CO_2 or decrease in the tension of O_2 in the respiratory centre, although it is possible that in extreme cases such changes may be of minor importance (Meakins and Davies (1925), Fraser (1927), Calhoun, Cullen, Harrison. Wilkins and Tims (1931), and Harrison, Harrison. Calhoun and Marsh (1932)).

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b. Increased ventilation is frequently included among the " causes " of cardiac dyspnoea. Since there is no signicant increase in the basal metabolic rate, the increase in ventilation, which runs pari passu with the degree of respiratory distress, is a sign rather than a cause of cardiac dyspnoea (Peabody, Wentworth and Barker (1917), Harrison, Harris and Calhoun (1931), Harrison, Harrison, Calhoun and Marsh (1932)).

c. Increased venous pressure has recently been suggested as the cause of cardiac dyspnoea (Harrison, Harrison, Calhoun and Marsh (1932). "Reflex stimulation of respiration because of increased pressure in the right side of the heart and in the cardiac ends of the great veins " is regarded as of especial importance in the genesis of cardiac dyspnoea. The evidence is based on the occurrence of rapid breathing in dogs due to two procedures, both of which raise the pressure in the right auricle. This twofold response can be obtained by injecting suddenly a large quantity of fluid into the venous system. Although rapid breathing results, there are many other systemic changes which occur besides a rise in the venous pressure. Cloetta and Stäubli (1919) have shown that the pressure in the pulmonary artery also rises under these circumstances and that, if the pleural cavities be open to the atmosphere, the volume of the lungs increases, or if this be prevented the functional residual air decreases. This can only mean an engorgement of the pulmonary capillaries resulting in the " Lungenstarre " of von Basch (vide infra). The same rigidity of the lungs is always found in congestive heart failure and can be produced by many experimental procedures such as multiple pulmonary emboli and anaphylaxis, which also result in rapid and shallow breathing and a rise in venous pressure. The relationship of pulmonary rigidity to cardiac dyspnoea will be discussed in greater detail below. The second method employed by Harrison, Harrison, Calhoun, and Marsh, was the inflation of a rubber balloon inserted into the right auricle of a dog. It was necessary to inject over 15 cc. of air into the balloon to obtain a significant increase in the respiratory rate. But they found that even passive movement of a leg may lead by a reflex mechanism to marked respiratory acceleration. The pain of coronary spasm is proverbial and the possibility that the reflex respiratory acceleration which they observed after such an insult to the auricular wall was due to a pain or shock reflex, should be considered.

d. The diminution of the vital capacity has long been known to be approximately proportional to the degree of respiratory distress. All the evidence indicates that diminution is due not to reflex inhibition of inspiration but to decreased distensibility of the lungs (Romanoff (1911), Meakins and Davies (1925), Hofbauer (1925)). No case in which the volume of the vital capacity did not exceed the limits of a normal respiration has been reported; the loose statement, that diminution in the vital capacity is a cause of cardiac dyspnoea, should cease to be repeated. The decreased distensibility of the lungs may increase the amount of work which must

be done to yield a breath of ordinary depth, or in some way may change the sensitivity of the Hering Breuer reflex, but these changes cannot be ascribed to a diminution of the volume of the vital capacity. The relationship of pulmonary distensibility to the Hering Breuer reflex and cardiac dyspnoea will be discussed later.

Orthopnoea has been ascribed to the same factors as have been described in the case of cardiac dyspnoea (Calhoun, Cullen, Harrison, Wilkins and Tims (1931), Weiss and Robb (1933)). It is evident that changes in aeration of the respiratory centre cannot be responsible for orthopnoea. Weiss and Robb conclude that " changes in vital capacity and lung volume are to be regarded as the most important causes of orthopnoea." In other words, orthopnoea is in some way linked with the distensibility of the lung, which has long been known to vary with posture. The relationship of pulmonary distensibility to orthopnoea will be discussed later in greater detail.

Paroxysmal cardiac dyspnoea (cardiac asthma) is closely associated with signs of acute pulmonary congestion and is evidently also caused by a sudden decrease in pulmonary distensibility, from the intense engorgement of the pulmonary capillary bed (Weiss and Robb (1933)).

Cheyne-Stokes breathing is presumably of central origin (Meakins and Davies (1925); Anthony, Cohn and Steele (1932)) and will not be considered further in this communication.

From the evidence presented in this brief review it is evident that changes in distensibility of the lungs are probably of prime importance in the causation of cardiac dyspnoea, orthopnoea and cardiac asthma. The elastic properties of the lungs in heart failure have never been subjected to experimental analysis. In vivo, they have not been measured, and the validity of postmortem observations on the distensibility of the lungs is open to question (Christie and McIntosh (1934)). The methods employed in this investigation have already been fully described (Christie and McIntosh (1934), Christie (1934)).

RESULTS

It has been shown (Christie and McIntosh (1934)) that the elastic properties of a lung can be defined in considerable detail by simultaneous registration of the tidal air and intrapleural pressure. The elasticity of the normal lung is very nearly perfect and its distensibility falls within a comparatively narrow range. An attempt has been made to obtain a record of the intrapleural pressure in seven cases of congestive heart failure. In two, free pressure fluctuation from the pleural space was not obtained. The significance of the failure in these two cases will be discussed later, but in the other five, tracings which cannot be considered normal were secured.

I. Pulmonary distensibility

Instead of negative intrapleural pressure throughout the respiratory cycle, the pressure rises at the end of expiration to that of the atmosphere, or higher (Figures I to VII and Figure IX). Any measure of distensibility involves the force applied to distend the lungs. In cardiac failure it is evident that some of the distension during inspiration is consequent
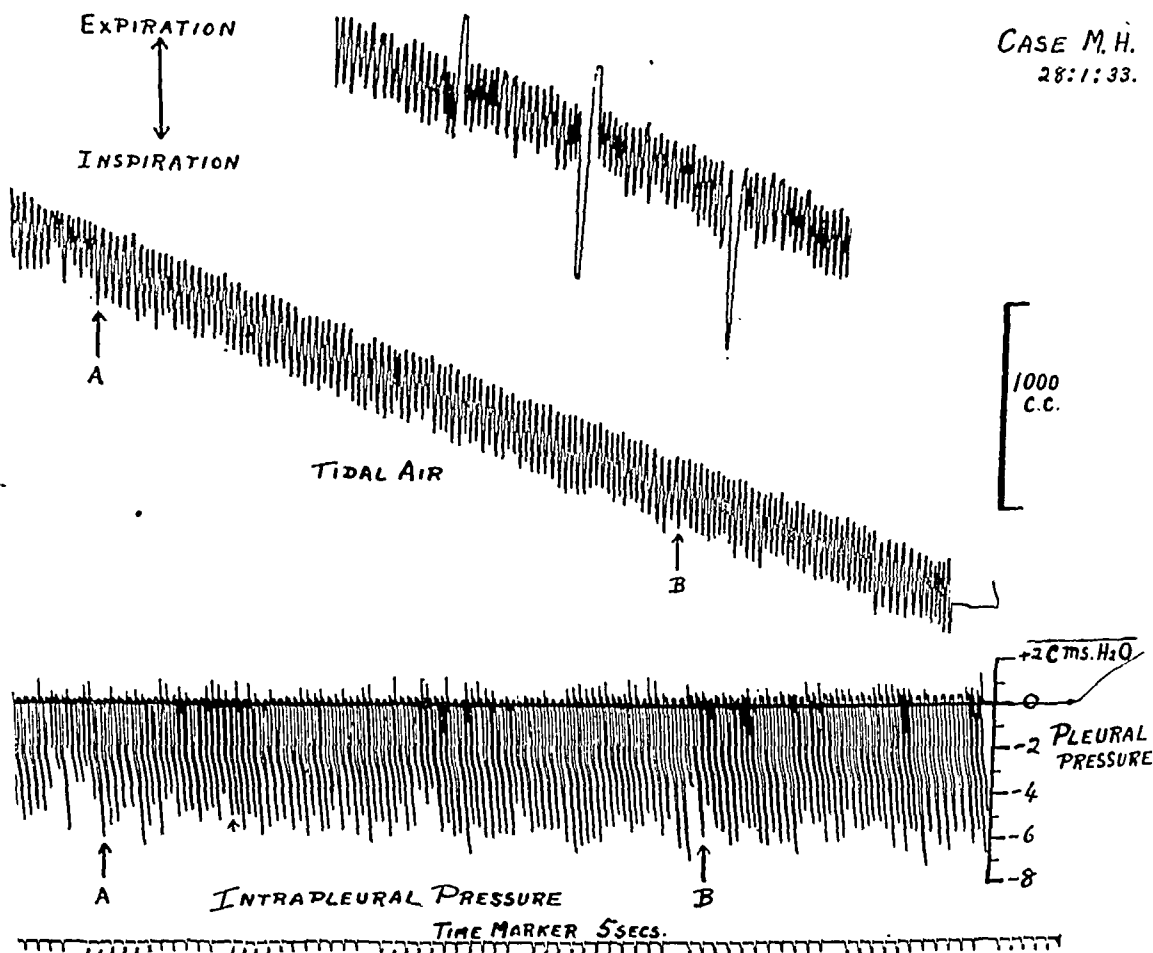
FIGURE I. THE INTRAPLEURAL PRESSURE IN CONGESTIVE HEART FAILURE

Case M. H. The upper tracing is of the vital capacity, and complemental and reserve air taken separately. The middle tracing is of the tidal air taken synchronously with the intrapleural pressure (lower tracing).

on the release from positive pressure applied during expiration. This part reflects the compressibility of the lungs rather than the distensibility. Even in the case of a sponge of perfect elasticity, compressibility, if expressed quantitatively, may be very different from distensibility. Unfortunately, it is impossible to measure the compressibility of normal lungs, since in health they always deflate by elastic recoil; but in a subsequent communication on artificial pneumothorax, we will show that, with the lung partially
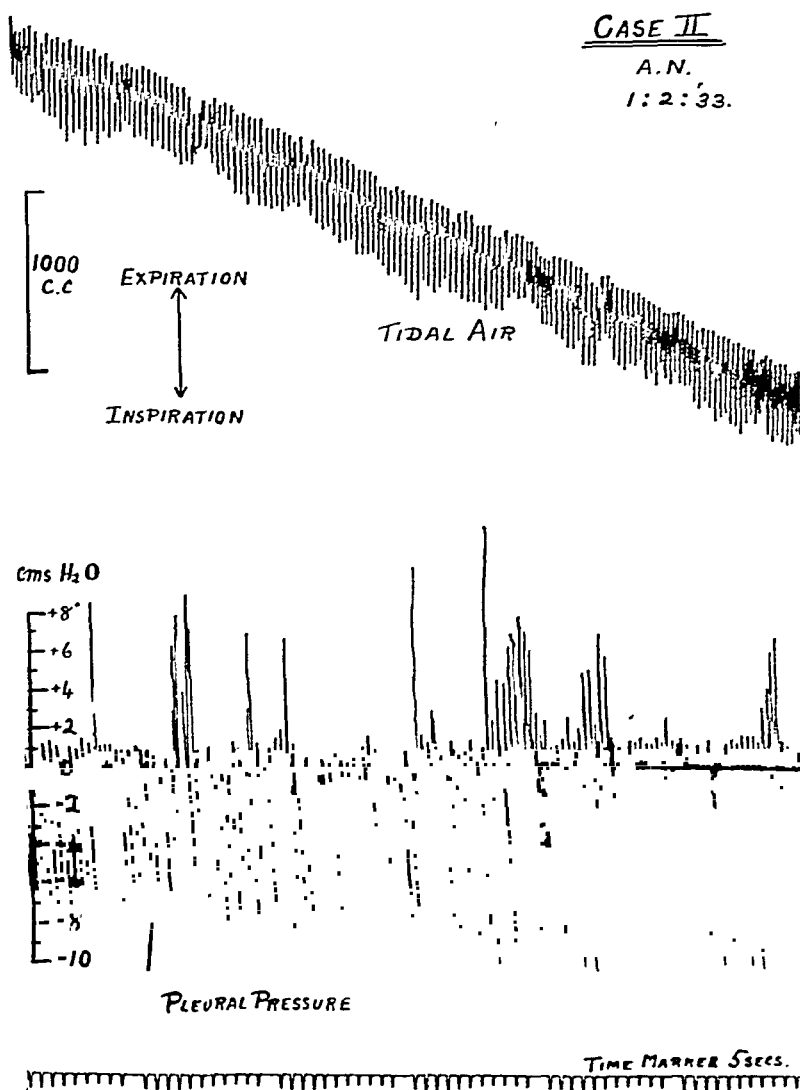
FIGURE II. THE INTRAPLEURAL PRESSURE IN CONGESTIVE HEART FAILURE

Case A. N.   The upper tracing is of the tidal air taken synchronously with the intrapleural pressure (lower tracing).   The expiratory irregularities in the intrapleural pressure are due to coughing and grunting.
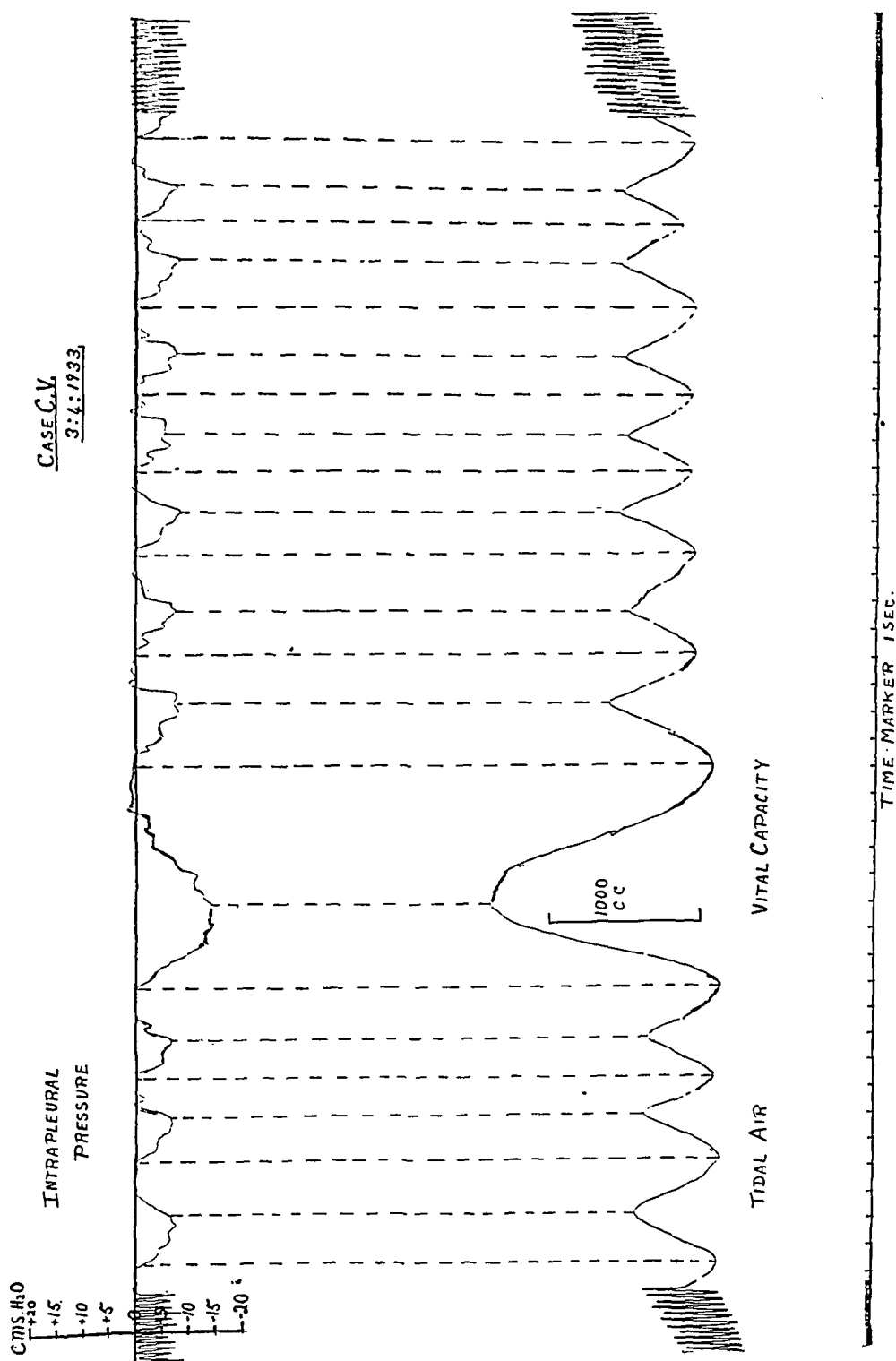
FIGURE V. THE INTRAPLEURAL PRESSURE IN CONGESTIVE HEART FAILURE

Case C. V. The lower tracing is of the tidal air taken synchronously with the intrapleural pressure (upper tracing). With a fast moving drum the correspondence between the maximum degree of distension when a deep breath is taken, and the point of greatest negativity in the intrapleural pressure is well shown.
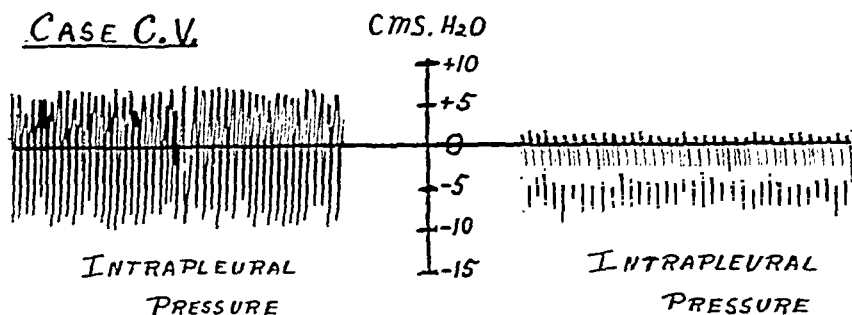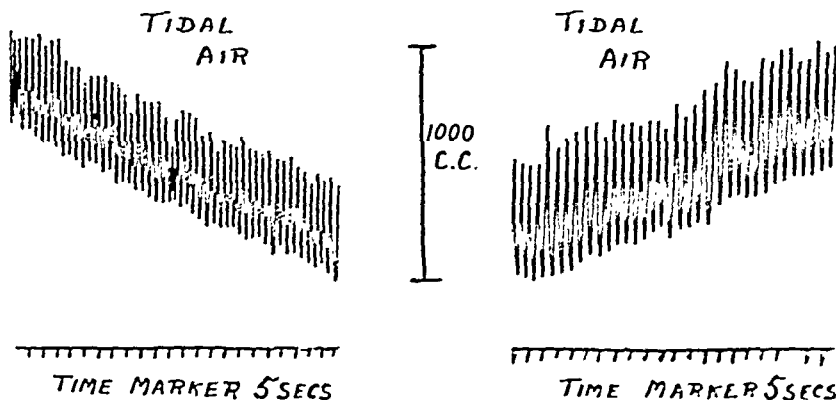
FIGURE VI. THE INTRAPLEURAL PRESSURE AND ITS RELATIONSHIP TO THE DEGREE OF CONGESTIVE HEART FAILURE

Case C. V. After 6 weeks' rest in bed and treatment there is a diminution in the intrapleural pressure fluctuation, even with an increase in the tidal air.
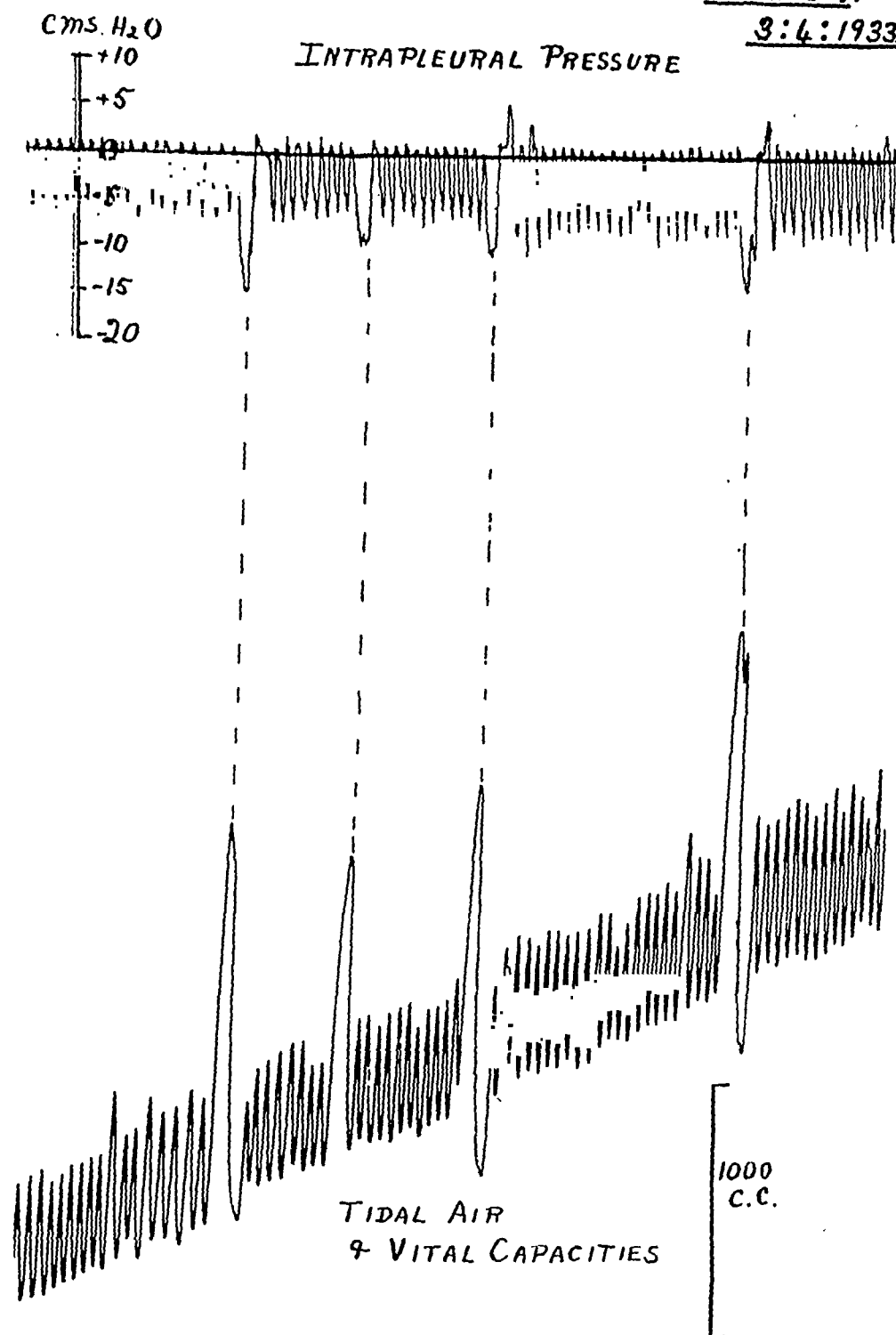
FIGURE VII.   THE INTRAPLEURAL PRESSURE AND ITS RELATIONSHIP TO PUL-
MONARY DISTENTION IN CONGESTIVE HEART FAILURE

Case C. V.   Any increase in the depth of inspiration is accompanied by a
corresponding decrease in the intrapleural pressure.

collapsed, three or four times the force is required to compress the lung as is required to distend it. From this it is obvious that a measure of the distensibility of the lung should only include the negative pressure exerted and only that portion of the inspiration which results from the application of negative pressure. Unfortunately, it is impossible in cardiac failure to distinguish that part of inspiration which is the result of the expanding force. To make this distinction in emphysema is unnecessary, for the intrapleural pressure returns to that of the atmosphere at the end either of a deep inspiration or expiration. Such a loss of elasticity is not evident in heart failure (vide infra). No more is possible than to calculate distensibility on two assumptions; first, that there is no elastic recoil from compression; and second, on the assumption that the compressibility and distensibility are equal and that elastic recoil from compression is perfect. In the former case only the negative pressure exerted on the lungs constitutes the expanding force, while in the latter the total intrapleural pressure fluctuation is included. Both calculations are erroneous; the true figure lies between them (Table I). With either method of calculation the coefficient

TABLE I

Pulmonary distensibility in congestive heart failure

Case	Functional residual air	Distensibility in cm. of H_2O per 100 cc. distension		Coefficient of distensibility *	
		From total pressure exerted	From negative pressure exerted	From total pressure exerted	From negative pressure exerted
	cc.				
S.S.	2905	3.24	2.89	18.8	16.7
M.H.		2.07	1.90		
J.P.	1150	8.78	5.43	20.2	12.5
A.N.	2315	3.09	2.7	14.3	12.5
C.V.					
(February 22, 1933)		3.76	2.21	19.0	11.2
(April 3, 1933)	2530	1.67	1.59	8.45	8.05
Normal				3.5 to 6.0	3.5 to 6.0

* Coefficient of distensibility = The force in cm. of H_2O required to distend the lung by 20 per cent of the functional residual air.

of distensibility is greatly increased. Symptomatic improvement is accompanied by a fall in this coefficient (Case C. V.). The conclusion is drawn from these figures that there is profound impairment of distensibility in the lungs in heart failure with congestion and that this impairment diminishes with symptomatic improvement.

II. Pulmonary elasticity

In Figure VIII the degree of distension is plotted against the force exerted on the lung. In the upper curve this force is taken as the total pressure fluctuation, while with the middle curve only the negative pressure applied is included. The two curves are not parallel but approach one another as the depth of inspiration increases (Figure VIII). The conclusion to be drawn is that release from positive pressure results in a comparatively small amount of pulmonary expansion. The middle curve is then a truer representation of the distensibility than the upper and, although its real position probably lies somewhat higher on the scale, its slope must be representative of the proportionality between stress and strain. It has been shown (Christie and McIntosh (1934)) that in the healthy lung the stress is proportional to the strain but in this case there is a slight increase in distensibility as the depth of breathing increases. This can only mean some impairment of pulmonary elasticity, but an impairment which is small compared with that observed in emphysema (Christie (1934), Figure I). The cruder tests of pulmonary elasticity which have been described (Christie and McIntosh (1934)) fail to reveal any elastic impairment in congestive heart failure. If a deep breath be taken, the point of maximum negativity of the intrapleural pressure corresponds closely to the height of inspiration (Figure IV and V). We have been unable to obtain a record of inspiratory apnoea with the glottis open, but when the vital capacity is measured no " set " can be demonstrated in cases uncomplicated by true emphysema (Figure I). Case S. S. did have signs of mild emphysema. His reserve air averaged 325 cc., when taken alone, and 270 cc., when taken after a deep inspiration. He also shows the increase in functional residual air (Figure IX) and irregularity of the respiratory level (Figure III), which are characteristic of emphysema.

We conclude from these measurements that there is a slight loss of pulmonary elasticity in congestive failure.

III. The lung volume and its subdivisions

There is a decrease in the volume of the vital capacity and a relative increase in the functional residual air (Figures IX and X), (Binger (1923), Meakins and Christie (1929)). The diminution, or even obliteration, of the reserve air is also clearly shown (Hofbauer (1925), Meakins and Christie (1929)). The validity of the lung volume measurements made by Rubow (1908), Bittorf and Forschbach (1910), Siebeck (1912), Peters and Barr (1920) and Lundsgaard and Schierbeck (1923) are open to question and have been discussed elsewhere (Christie (1932)).

IV. Haemo-respiratory exchange

With diminution of the reserve air, which is characteristic of this condition, any measurement of the alveolar air by the Haldane-Priestley
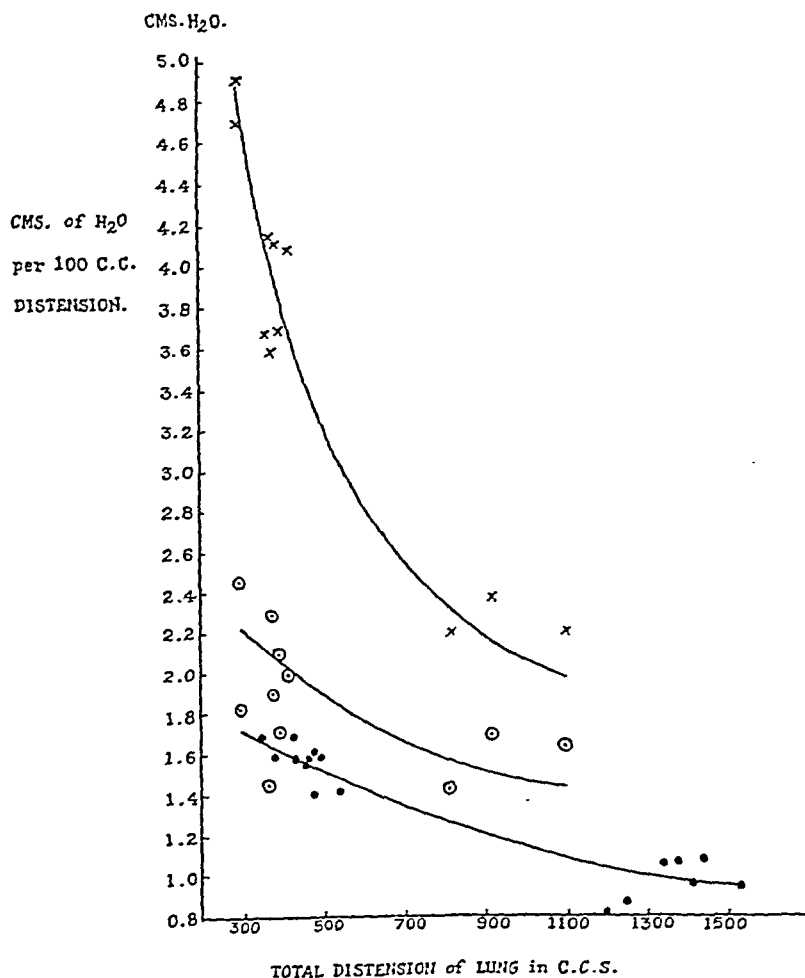
FIGURE VIII. THE PULMONARY DISTENSIBILITY IN CONGESTIVE HEART FAILURE

Case C. V.

X = Depth of inspiration plotted against

$$\frac{\text{Total change in intrapleural pressure}}{\text{Depth of breath in cc.}} \times 100.$$

⊙ and · = Depth of inspiration plotted against

$$\frac{\text{Negative pressure change in intrapleural pressure}}{\text{Depth of breath in cc.}} \times 100.$$

X and ⊙ = From measurements taken on February 22nd, 1933.

· = From measurements taken on April 3rd, 1933, after 6 weeks' rest in bed and treatment.
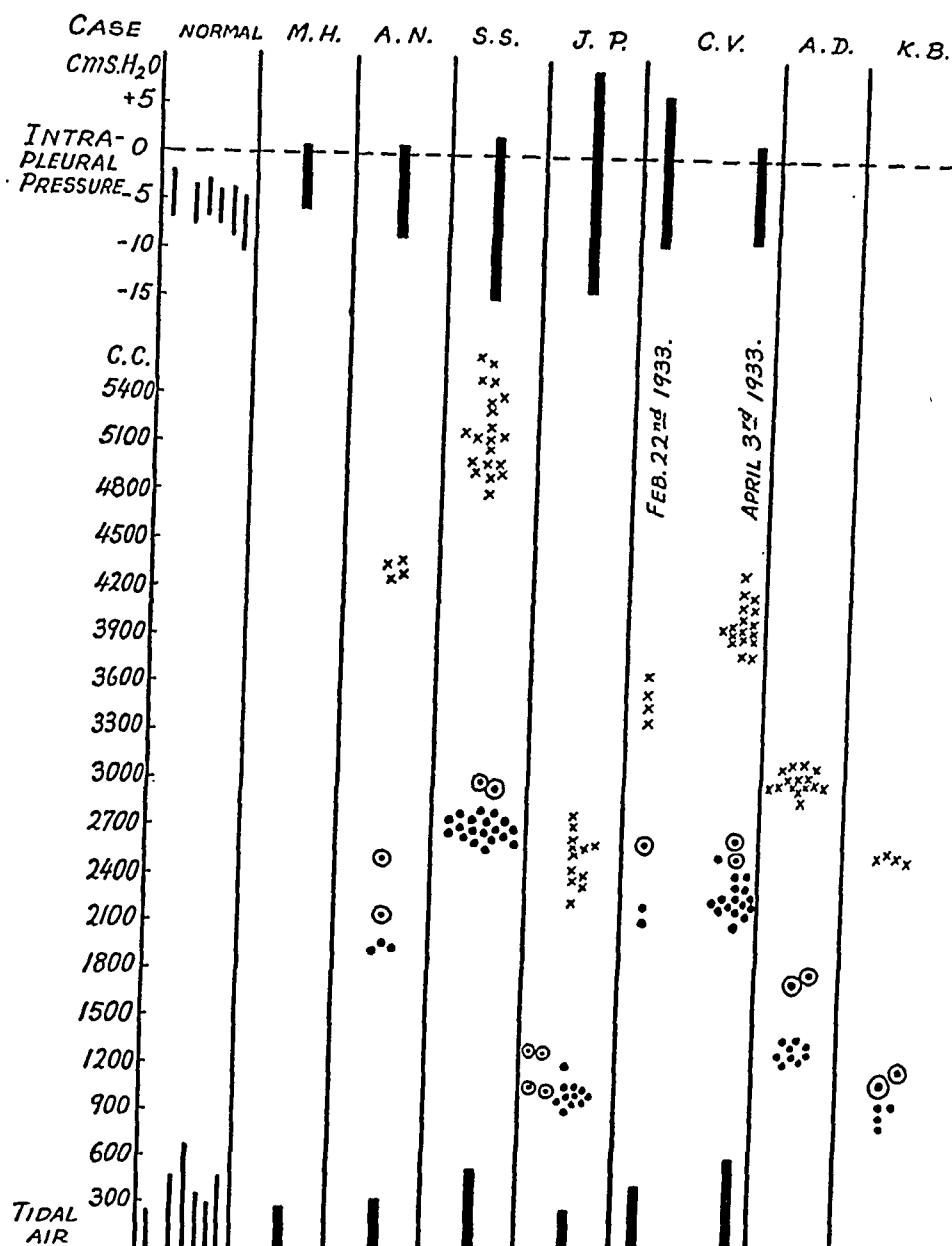
FIGURE IX.   THE LUNG VOLUME AND ITS SUBDIVISIONS IN CONGESTIVE HEART
FAILURE

X = Total capacity
⊙ = Functional residual air
• = Residual air.

The average intrapleural pressure fluctuation with the corresponding tidal air is shown on 5 cases of congestive failure and 5 individuals with minimal apical tuberculous lesions, with functional residual air ranging from 1300 to 3775 cc.
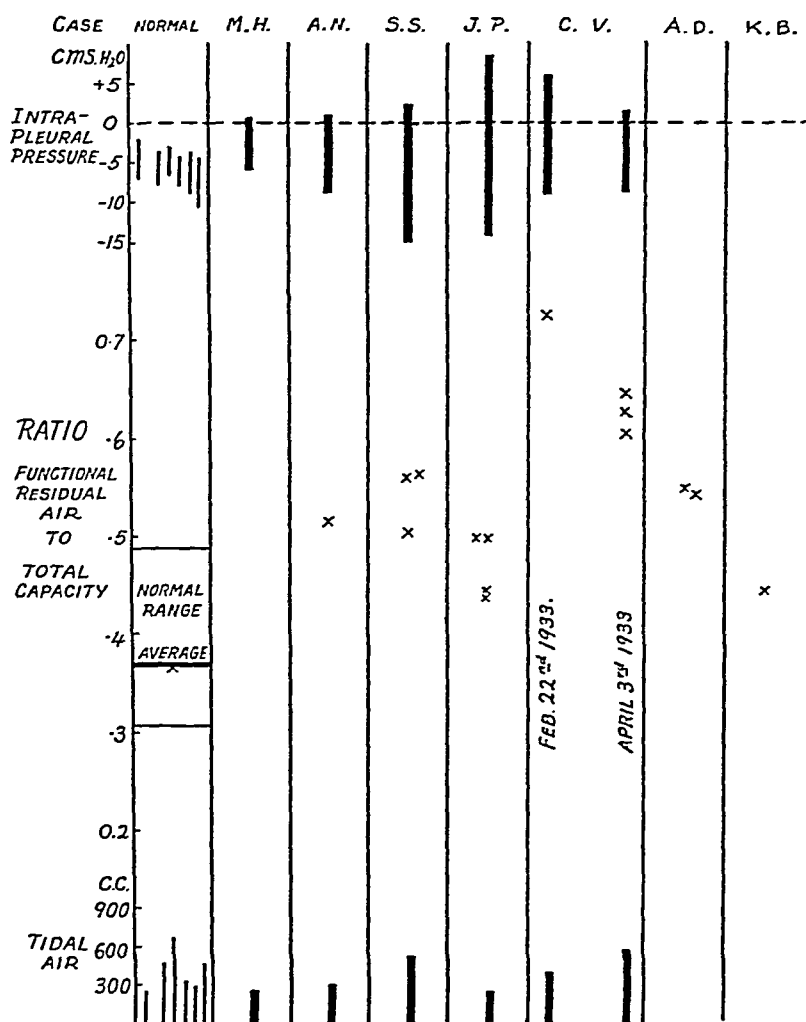
FIGURE X.   THE RATIO OF FUNCTIONAL RESIDUAL AIR TO TOTAL CAPACITY IN CONGESTIVE HEART FAILURE

$$X = \text{ratio} \quad \frac{\text{Functional residual air}}{\text{Total capacity}}$$

The average intrapleural pressure fluctuation with the corresponding tidal air is shown as in Figure VIII.

method must be of doubtful significance. What reserve air can be expelled in heart failure is expelled slowly and with difficulty, so that, even if an " inspiratory Haldane-Priestley sample " be taken, the respiratory quotient may be extremely low. It was this type of sample that was secured in Cases A. N., S. S. and C. V. (Table II). The respiratory quotient in all

TABLE II

Haemo-respiratory exchange in congestive heart failure

Case	Tidal air	Respiratory rate per minute	O₂ consumption per minute	Alveolar pCO₂ (Haldane Priestley)	Alveolar pCO₂ (automatic sampler)	Expired pCO₂	Arterial blood		
							O₂ capacity	O₂ saturation	pCO₂
	cc.		*cc.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*volumes per cent*	*per cent*	*mm. Hg*
A.N.	314	21.2	247	35.5	32.1	19.6	19.75	90.1	36.5
S.S.	687	12.7	344	37.1	28.4	23.6	24.59	94.0	27.0
C.V.	319	15.8	209.5	37.8	29.4	20.0	15.66	89.9	31.0
J.P.	234	23.5	209	26.2	23.3	13.1			
M.H.							21.81	89.5	37.5
A.D.	174	31.2	185.0			18.3	13.66	95.9	36.5

these cases was below 0.6, so that the samples were probably more nearly in equilibrium with the mixed venous blood than with the arterial blood. In Case J. P. it was 0.75 (in the expired air it was 0.83), but with complete absence of reserve air, the sample probably consisted largely of dead space air. When the automatic sampler was used, the respiratory quotient always corresponded with that of the expired air and we believe that, by this method, the pressure of CO_2 reflects more closely the pressure in the alveolar air. Such a supposition is supported by the pressures found in the arterial blood.

The oxygen saturation of the arterial blood was always below normal but in no case was sufficient anoxaemia present to account for the presence of dyspnoea.

In all our cases, including S. S., a case of slight emphysema, the haemo-respiratory picture is typical of congestive heart failure (Meakins and Davies (1925); Fraser (1927); Calhoun, Cullen, Harrison, Wilkins and Tims (1931); and Harrison, Harrison, Calhoun and Marsh (1932)).

DISCUSSION

I. Cardiac dyspnoea

In 1891 von Basch described the rigidity ("Lungenstarre") of the lung in heart failure with congestion. He and his associates showed at autopsy that the lungs were less distensible than normal and suggested that in this mechanical limitation of inspiration lay the cause of cardiac dyspnoea.

This postmortem change has been confirmed by many investigators, some of whom have suggested that, instead of a mechanical limitation of inspiration, some disturbance of the Hering Breuer reflex is responsible for the respiratory embarrassment (Meakins and Davies (1925), Hofbauer (1925), Field and Bock (1925–26), Anthony (1930), Fraser (1927), Harrison, Calhoun, Cullen, Wilkins and Pilcher (1932)). The inference which has been drawn from postmortem observations on the degree of distensibility of the lungs is open to question (Christie and McIntosh (1934)), but we have been able to confirm the observations of von Basch in every respect by measurements made in vivo. Experimental pulmonary congestion (Churchill and Cope (1929)) and other conditions, such as multiple pulmonary embolism (Moore and Binger (1927)), which increase the rigidity of the lung, lead to reflex rapid and shallow breathing which is abolished by vagal section. It is reasonable to suppose that the same mechanism may be responsible for cardiac dyspnoea.

We have already emphasized our belief that all the direct evidence in the literature indicates that cardiac dyspnoea is in some way associated with changes within the lung, rather than with central stimulation. The only demonstrable change in the lungs which can be linked with respiration is decrease in distensibility. It has been suggested that stiffness of the lungs mechanically limits the volume of the tidal air in the same way as it limits that of the vital capacity. In our studies on artificial pneumothorax, where the vital capacity can be slowly reduced to a volume smaller than is found in most cases suffering from cardiac dyspnoea, there has been found no evidence that respiratory distress exists even on mild exercise. There is ample evidence, however, that certain experimental procedures which increase the rigidity of the lung lead reflexly to rapid and shallow breathing. The concensus of opinion still centers on the idea that the Hering Breuer reflex is dependent on change in tension within the lungs. It seems reasonable to suppose that cardiac dyspnoea is largely, if not wholly, dependent on increased sensitivity of this reflex, resulting from the decreased pulmonary distensibility.

Orthopnoea seems in the same way to be linked with some change in the lungs due to posture, though the relation of changes in posture to changes in pulmonary distensibility is less obvious. A simple explanation was given by Hill in 1895, in the course of an inquiry into the effect of gravity on the circulation of the blood (Field and Bock (1925–26)). After having made other observations, which have since become commonplaces, he suggests that the upright position relieves orthopnoea by draining blood into the splanchnic area (and liver). Field and Bock (1925–26) have observed diminution in the rate of blood flow in the upright position confirmed by Bielschowsky and others (Bielschowsky, 1932)) and believe this to be due directly to an effect of gravity in impeding the return of blood to the heart. They discuss the occurrence of stagnation of blood in the

use of an abdominal belt, is due to facilitation of venous return by the increase in intraabdominal pressure.

V. Emphysema in congestive heart failure

With the decrease of distensibility which has been demonstrated in congestive heart failure, the alveoli are subjected to increased tension change with inspiration and expiration. It is not unreasonable to suppose that these surface alveoli might readily be overstretched and suffer a further loss of elasticity with the production of emphysema. The high incidence of emphysema in cases with chronic congestion has frequently been noted. It is this mechanism, so we believe, which leads to the emphysema of high altitudes. There is ample evidence in the literature to show that at high altitudes the lungs are congested and that emphysema develops. In unpublished investigations we have been able to demonstrate that the lungs of rats, acclimatized to a height of 18,000 feet, show a marked decrease in distensibility and that emphysema, mainly at the peripheral portion of the lungs, progressively develops.

SUMMARY AND CONCLUSION

1. From simultaneous tracings of the volume of the tidal air and of intrapleural pressure in heart failure with congestion, a marked decrease in the distensibility and slight impairment of the elasticity of the lung can be demonstrated.

2. Characteristic changes in the volume of the lungs are described; an explanation for these changes is advanced, on the basis of a decrease of pulmonary distensibility.

3. An analysis of haemo-respiratory exchange does not yield an adequate explanation of the functional impairment in this type of heart failure.

4. Evidence is presented, which rests on the basis of impairment of pulmonary distensibility and elasticity, that the peripheral alveoli are overventilated at the expense of the deeper alveoli. The relative importance of this and other mechanisms which occasion anoxemia accompanied by increased excretion of CO_2 is described.

5. Expiration in congestive heart failure is shown to be, in part, the result of positive intrapleural pressure generated by active muscular effort. The functional drawbacks of this type of expiration are emphasized, in particular diminution in the excursion of the diaphragm, with consequent impairment of inspiratory efficiency.

6. An increase of intra-abdominal pressure by means of an abdominal belt is of value in the treatment of heart failure with congestion. The rationale of this form of treatment is described.

7. Cardiac dyspnoea and orthopnoea can both be fully explained, on the basis of increased sensitivity of the Hering Breuer reflex due to changes in the distensibility of the lungs.

8. The significance of increased intrapleural pressure, in its relationship to venous pressure and the return of venous blood to the heart, is emphasized.

9. Decreased pulmonary distensibility, as a causative factor in the production of emphysema, is described, and an explanation for the high incidence of emphysema in heart failure and at high altitudes, advanced.

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APPENDIX

Case histories

M. H.—Female—Aet. 59.

Palpitation and dyspnoea on exertion for 2 years. Slight orthopnoea. Rheumatic fever in childhood. Blood pressure 175/100 with obvious auricular fibrillation. Arteriosclerosis. Heart rate 95 to 110. Transverse diameter of heart 14.5 cm. Heart-thorax ratio 0.59. No valvular disease. Percussion note impaired at bases with numerous moist râles. Liver and spleen not palpable. No oedema.

Diagnosis: Hypertension with auricular fibrillation and early congestive heart failure.

A. N.—Male—Aet. 25.

Palpitation and dyspnoea on exertion progressively getting worse during past 5 years. Scarlet fever in childhood. Obvious mitral stenosis with rough presystolic and soft diastolic and systolic murmurs. Transverse diameter of heart 16.3 cm. Heart-thorax ratio 0.6. X-ray of heart and electrocardiogram typical of mitral stenosis. Occasional moist râle at either base. Liver and spleen 1 finger's breadth below costal margin. No oedema.

Diagnosis: Mitral stenosis and regurgitation with early congestive heart failure.

S. S.—Male—Aet. 44.

Shortness of breath on exertion and swelling of ankles of 3 months' duration. Some orthopnoea for 2 to 3 weeks. Heart markedly enlarged. X-ray— Greatest transverse diameter 21.3 cm. Oblique diameter 21.5 cm. Transverse aorta 7.0 cm. Heart-thorax ratio 0.61. No valvular disease. Blood pressure 210/120. Occasional moist râle at right base. Liver 3 fingers' breadth below costal margin.

Diagnosis: Hypertension and congestive heart failure.

J. P.—Female—Aet. 22.

"Heart Trouble" with breathlessness on exertion since rheumatic fever 7 years ago. Since a miscarriage 8 months ago has had progressively increas-

ing weakness, breathlessness on exertion, orthopnoea and oedema of legs. Now sleeps in chair. Slight cyanosis. Obvious signs of mitral and aortic stenosis and regurgitation with auricular fibrillation. Transverse diameter of heart 19.5 cm. Heart-thorax ratio 0.675. Blood pressure 120/60. Moist râles at either base. Marked ascites and oedema of legs and back. Liver 4 inches below costal margin.

Diagnosis: Chronic myocarditis and endocarditis, mitral and aortic stenosis and regurgitation, auricular fibrillation and very advanced congestive heart failure.

C. V.—Male—Aet. 61.

February 22nd, 1933. Weakness and fatigue for the past 5 years. Dyspnoea on exertion, orthopnoea and swelling of legs, 6 months' duration. Swelling of abdomen 2 weeks' duration. Slight cyanosis. Some arteriosclerosis. Signs of aortic regurgitation with cardiac enlargement. Blood pressure 164/64. Transverse diameter 19.2 cm. Heart-thorax ratio 0.62. Moist râles at either base. Some fluid in both pleural cavities. Liver enlarged and pulsating. Ascites and oedema of back and legs. Blood Wassermann + + +.

April 3rd, 1933. With rest in bed and mild antiluetic and digitalis treatment, patient improved markedly. Oedema, ascites, dyspnoea and general condition much improved.

Diagnosis: Arteriosclerosis, syphilitic aortitis, aortic insufficiency, advanced congestive heart failure.

A. D.—Female—Aet. 38.

Dyspnoea and palpitation on exertion, anginal-like pains and swelling of ankles—2 years' duration. Typical signs of mitral stenosis and regurgitation. Heart 4½ cm. to right, 7½ cm. to left. Blood pressure 148/120. Moist râles at both bases. Liver and spleen 1 to 2 fingers' breadth below the costal margin. No oedema.

Diagnosis: Mitral stenosis and regurgitation with congestive heart failure.

K. B.—Female—Aet. 32.

Palpitation, dizziness and dyspnoea on exertion since delivery of 4th child, 8 months ago. Slight orthopnoea. Questionable cyanosis. Signs typical of auricular fibrillation with mitral stenosis and regurgitation. Blood pressure 95/80. Heart rate 87. Venous pulsation in neck. Transverse diameter of heart 17.4 cm. Heart-thorax ratio 0.7. Moist râles at either base. Liver one hand's breadth below costal margin. Spleen just palpable. No oedema.

Diagnosis: Chronic myocarditis with auricular fibrillation, chronic endocarditis with mitral stenosis and insufficiency, congestive heart failure.

THE LIPEMIA OF PREGNANCY

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It is well known that the blood serum of pregnant women often has a milky appearance. Various names have been applied to the condition, "apparence légèrement laiteuse," "adipositas sanguinas" and more recently the lipemia or lipoidemia of pregnancy. Based on little information concerning the nature of the condition, the early conjectures as to its cause have now only historic interest. Hewson thought it due to fat absorption, Puzos that blood was mixed with milk for the nourishment of the fetus, and John Hunter that it represented poorly assimilated chyle (1). Virchow in 1847 (2) demonstrated that the milky appearance was due to the presence of fat which he proved by shaking the serum of women in the last months of pregnancy, the " Hochgravide," with ether, thereby extracting fatty material. In 1845 Becquerel and Rodier (3) had produced evidence suggesting there also occurred an increase in phosphorus containing fats and variations in blood cholesterol. The way was prepared for application of more modern methods of blood lipid analysis.

These modern chemical studies began in 1911 with the demonstration that there occurs an increase in blood cholesterol during pregnancy. Priority for this discovery is usually accorded to Chauffard, Laroche and Grigaut (4). Actually, as they acknowledge, their work was but a confirmation by more exact analysis of the conclusions reached by Neumann and Herrmann (1) earlier in the same year. The latter authors subsequently (5) augmented their preliminary work by publishing the first complete analysis of the blood lipids in pregnancy. This second analysis, which included quantitative extraction and estimation of total fat, free and ester cholesterol, lipoid phosphorus and neutral fat, was performed on 5 litres of pooled blood collected from the bleeding which normally occurs from the uterus following parturition. The necessity of using large amounts of blood proved a distinct deterrent to such studies. Introduction of micro-methods by Autenrieth-Funk, Grigaut, Weston and more especially Bloor, has overcome this difficulty and made it possible to obtain enough blood by simple venipuncture. A complete analysis similar to that of Herrmann and Neumann above (5) may now be quite readily performed on 2 cc. of blood.

cent while free cholesterol is little changed, resulting in a smaller rise in total cholesterol (8 per cent). Phospholipid may or may not be found changed though on the average it is slightly higher in pregnancy (14 per cent). Thus a study of whole blood reflects few significant changes in fat metabolism during pregnancy.

Analyses of *blood plasma* and *serum* have produced more important evidence of an altered fat metabolism. Fewer studies have been made on plasma or serum than on whole blood and only two (15, 20) have included simultaneous estimations on both. Hellmuth (33) and Oser and Karr (20) have reported the lipid content of the red blood cells in addition to that of serum or plasma.

Blood lipids are not equally distributed between cells and plasma. The relationship between plasma lipids and the erythrocytes is imperfectly understood. It would appear that plasma transports lipids to and from the body tissue while the red cells may be considered to act as temporary storehouses for fatty substances. Compared with plasma they play a minor rôle in fat metabolism. It is evident that more suggestive data relative to the lipemia of pregnancy may be obtained from analysis of plasma or serum than from analysis of whole blood. Simultaneous examination of both plasma and whole blood affords complete information and is the procedure of choice.

The cholesterol content of plasma and serum has been studied more extensively than other lipids. Chauffard, Laroche and Grigaut (4) were the first to demonstrate an increased total cholesterol in serum, a discovery later corroborated by various authors (28 to 36) including the recent nephelometric study of Kaufmann and Mühlbock (35). It has likewise been found increased in plasma by Slemons and Stander (15) with Bloor's methods, similarly by Oser and Karr (20) and later by Gardner and Gainsborough (37) with the digitonin method. Lower absolute values are obtained for cholesterol by the digitonin method than by colorimetric methods, due probably to the fact color is produced in the Liebermann-Burchard reaction by substances in blood other than cholesterol and also possibly because digitonin may not precipitate modified forms of cholesterol which are detected by sulphuric acid and acetic anhydride in the colorimetric procedure. Digitonin precipitation is, however, regarded by most authors as the more exact chemical procedure, or, as the German authors would have it, the more " elegant."

Both free and ester cholesterol have been found increased in plasma and serum (15, 33, 34, 35, 37) in contrast to an increase only in ester cholesterol of whole blood. Gardner and Gainsborough (37) recorded extensive fluctuations in the ratio of free to ester cholesterol throughout pregnancy, a finding which was not substantiated by the recent work of Kaufmann and Mühlbock (35). The latter authors noted little variation in the ratio from the second month of pregnancy to term.

In 1923, Plass and Tompkins (38) and Slemons and Stander (15) demonstrated an increase in " lecithin " of serum and plasma during pregnancy in contrast again to no significant change in whole blood. This change was borne out by subsequent investigations (20, 33, 34). No values have been reported on the neutral fat of plasma in pregnancy, but Fahrig and Wacker (34) found an increase in serum and a similar increase may also be calculated from the published figures of Hellmuth (33). As noted below, a marked increase of 129 per cent was recorded for plasma glycerol fat during pregnancy by the author. Summing up these various augmentations in the individual lipids of plasma and serum, the total lipid content is some 50 per cent higher in pregnant women which again is much more than noted above in whole blood.

It may be seen that in plasma or serum there is a greater change than in whole blood. In plasma (Table II) the level of total lipids is on the average half as high again as in the non-pregnant and all the component lipids are increased. The greatest increase is in neutral fat which is more than double its non-pregnant level while phospholipid, free and ester cholesterol are each higher than in non-gravid women.

From the results on whole blood and plasma it is obvious that the fatty changes in the *red blood cells* during pregnancy must be of an entirely different nature from those in plasma. Such has indeed been found the case in the present analysis.

Determination of the lipid content of the red blood cells may be done in two ways, by direct analysis of extracts of the cells or indirectly by analysis of whole blood and plasma and, from the hematocrit, calculating the lipid content of the erythrocytes. The first method, while admittedly the superior, is fraught with many difficulties. Alcohol-ether extracts of red blood cells are very apt, if not invariably, to contain decomposition products of hemoglobin. These exhibit many of the qualities as to solubility and precipitation of lipids and are hard to separate from them. The same difficulties are associated with extracts of whole blood to a much lesser extent, but may be overcome by not being too vigorous in boiling the alcohol-ether during extraction. After trying direct extraction, the author has abandoned this in favor of the indirect method. The latter, if done carefully, will give results practically identical with those by the former procedure.

No complete analysis of the lipids in red blood cells during pregnancy has appeared in the literature. Oser and Karr (20) have recorded values for " lecithin " and total cholesterol while Hellmuth (33) has presented all values except neutral fat. This latter lipid may be calculated from his published figures. His values in three cases for total fatty acid of red cells 7 to 12 days postpartum is less than would account for the fatty acids of phospholipid present, to say nothing of the fatty acids from cholesterol ester and neutral fat, each of which are present in appreciable amounts

TABLE II

The fasting level of plasma lipids by oxidative micro-methods in non-gravid women and women at term in pregnancy. The results are expressed in mgm. per 100 cc. of plasma

Case number	Composition of total lipid							Iodine numbers		Lipid ratios	
	Total lipid	Total fatty acid	Neutral fat	Phospho-lipid	Cholesterol			Total fatty acid	Phospholipid fatty acid	$\dfrac{\text{Phospholipid}}{\text{Cholesterol}}$	$\dfrac{\text{Ester cholesterol}}{\text{Total cholesterol}}$
					Total	Ester	Free				
Non-pregnant											
1	504	281	83	195	154	107	47	124	109	1.27	0.69
2	520	289	99	195	161	97	64	89	77	1.21	0.60
3	551	319	123	198	158	105	53	83	133	1.25	0.66
4	607	340	125	184	200	147	53	96	121	0.92	0.73
5	630	398	205	179	162	125	37	58	128	1.10	0.77
6	655	404	219	156	188	143	45	77	126	0.83	0.76
7	655	349	55	293	206	151	55	92	138	1.42	0.73
8	675	388	162	181	219	168	51	88	125	0.83	0.77
9	752	493	317	176	184	110	74	63	142	0.96	0.60
Mean	617	362	154	195	181	128	53	86	122	1.09	0.70
Standard deviation	75	62	77	37	22	23	10	18	19	0.20	0.064
Pregnant											
1	684	460	287	196	145	83	62	84	115	1.35	0.57
2	717	474	291	207	159	89	70	66	103	1.30	0.56
3	843	593	412	186	167	117	50	100	167	1.11	0.70
4	879	594	374	231	189	127	62	122	148	1.22	0.67
5	896	565	299	252	232	168	64	78	96	1.09	0.72
6	943	628	365	276	205	145	60	77	122	1.35	0.71
7	1013	605	238	314	292	252	40	86	105	1.08	0.86
8	1061	740	488	284	202	130	72	78	93	1.39	0.64
9	1064	692	420	285	256	153	103	84	100	1.11	0.60
Mean	900	595	353	248	205	140	65	86	117	1.22	0.67
Standard deviation	130	85	75	43	45	47	15	15	24	0.12	0.088

according to his analysis. Be that as it may, he has shown that the red blood cells in pregnant women at term contain but slightly more lipids than those of non-pregnant women. Total lipid, neutral fat (calculated from his figures), "lecithin" and free cholesterol each, on the average, are slightly elevated in pregnancy while cholesterol ester, of which there are only traces in normal non-pregnant women, he records as high as 90 mgm. per. cent in certain cases. Oser and Karr report increases in "lecithin" and total cholesterol. In general, the lipids which have been studied in the red cells exhibited practically no noteworthy change during gestation. A complete analysis of these cells has been presented in Table III and it is

TABLE III

The fasting level of red blood cell lipids by oxidative micro-methods in non-gravid women and women at term in pregnancy. The results are expressed in mgm. per 100 cc. of cells

Case number	Total lipid	Composition of total lipid						Lipid ratios	
		Total fatty acids	Neutral fat	Phospholipid	Cholesterol			Phospholipid / Cholesterol	Ester cholesterol / Total cholesterol
					Total	Ester	Free		
Non-pregnant									
1	473	267	85	279	109	0	109	2.56	0.0
2	537	297	76	337	124	0	144	2.72	0.0
3	564	302	11	437	116	0	129	3.76	0.0
4	604	329	137	271	178	27	151	1.52	0.15
5	607	324	95	337	166	13	153	2.03	0.08
6	617	366	92	418	107	0	121	3.91	0.0
7	626	388	148	372	106	0	113	3.51	0.0
8	661	331	54	418	189	0	194	2.21	0.0
9	692	397	143	377	162	15	147	2.33	0.09
Mean	598	333	93	361	140	6	140	2.73	0.036
Standard deviation	62	41	42	56	32	9	26	0.78	0.053
Pregnant									
1	368	206	104	160	104	0	132	1.54	0.0
2	418	288	169	191	58	0	60	3.29	0.0
3	467	258	0	387	80	0	83	4.83	0.0
4	527	292	0	417	95	22	73	4.38	0.23
5	578	325	156	266	156	0	156	1.71	0.0
6	639	387	191	284	147	26	121	1.93	0.18
7	660	421	182	372	106	0	112	3.51	0.0
8	692	343	0	478	190	36	154	2.52	0.19
9	999	507	0	698	260	61	199	2.69	0.23
Mean	594	336	89	361	133	16	121	2.93	0.092
Standard deviation	123	86	80	155	62	21	43	1.10	0.104

obvious that no significant variation can be detected between pregnant and non-pregnant women.

Comparing the analyses on whole blood, plasma and red cells, it is apparent that the most important changes in the level of lipids during pregnancy occurs in blood plasma. The red cells exhibit little variation in their lipid content. And, to repeat, the characteristics of the altered level of lipids in plasma are that neutral fat is doubled in value, phospholipid, free and ester cholesterol are each increased and the normal ratios between these latter three lipids but slightly altered. Analysis of whole blood alone affords no evidence of these singular occurrences in the plasma.

EXPERIMENTAL PROCEDURE

The purpose of the present series of experiments was twofold. First to demonstrate experimentally that variations in the level of lipids during pregnancy were not the same in whole blood, plasma and red cells and by so doing to bring into harmony the apparent discrepancies in previous observations on the nature of the lipemia of pregnancy. Secondly, to establish a range of values for the various blood lipids in normal pregnant women by the more recent improved micro-methods. As described above the first premise has been aptly fulfilled and not only have the conclusions of previous investigators been synchronized, but several gaps in knowledge concerning one or another lipid in blood have been filled in. A complete picture of the lipemia of pregnancy has been presented. Having treated the qualitative changes, we may now proceed to a more exact consideration of the quantitative relationships and procedures used.

A number of women of various ages and parity were selected from the prenatal clinic of the Strong Memorial Hospital. From their records of medical history and physical examination they were diagnosed as normal, healthy, pregnant women approaching or within one month of the termination of gestation. In the earlier cases a complete analysis of whole blood, red cells and plasma was not obtained due to the necessity of discarding most of the early direct analyses of the red blood cells for reasons explained above. Having diverted from this procedure to the one reported below, nine cases were studied in detail and as these appear to clearly present all the necessary information it has not seemed advisable to include the earlier values.

As the expected date of confinement approached, each patient was referred to the obstetrical division of the hospital where a more thorough work-up was instituted to assure absence of conditions other than pregnancy which are known to affect the level of blood lipids as listed in a previous communication on the levels of blood lipids in non-gravid women (41). A few cases showing no abnormality except a mild albuminuria were subjected to the Van Slyke urea clearance test, Addis count and creatinine

clearance, in addition to the usual chemical studies of blood. In all cases, renal function was found to be normal. Similar criteria (41) were applied to nine normal non-pregnant women who served as controls for the above cases.

Having kept the patient in bed on a standard balanced diet for one week, blood was withdrawn from the arm veins at 8.00 to 9.30 A.M. following a 16 hour fast. The blood was shaken in a small flask, the interior of which had been coated with half-saturated sodium citrate. Five cc. were removed for analysis of whole blood and the remainder centrifuged for one-half to three-quarters of an hour. The values for lipids in the red blood cells were determined from those of the plasma and whole blood in conjunction with the hematocrit reading. The choice of a suitable anti-coagulant presented some difficulties. Richtner-Quittner (43) claimed that sodium oxalate and fluoride injure the red blood cells, allowing escape of certain of their lipid constituents into plasma; Gardner and Gainsborough (39) were unable to confirm this. Sodium citrate has been extensively used by Bloor and his associates. In the dry form or in saturated solution it has been occasionally noted by the author to produce slight hemolysis. When half saturated solutions were used in quantity sufficient to line the inside of the flask, which was allowed to drain before addition of blood, the reagent was found quite satisfactory.

The 5 cc. of blood removed for whole blood analysis were added to about 75 cc. of an alcohol and ether mixture, brought to boiling, filtered and made up to 100 cc. after the method of extraction reported before (41). Ten cc. of plasma obtained after centrifugalization were similarly extracted and made up to 250 cc., the larger volume of extract being necessary to assure sufficient lipid for micro-determination of iodine numbers. The alcohol-ether extracts were then analysed for their lipid content after the manner previously described in detail (41). Briefly, the general procedure is based on a number of oxidative micro-methods in which the lipid to be estimated is isolated by selective solubility or precipitation and then oxidized completely with chromic acid. The lipid content is then calculated from the amount of chromic acid required for this purpose. Iodine numbers were estimated on total fatty acids and phospholipid fatty acids of plasma. From the values determined directly, the remaining, as in Tables I, II, and III, were found by calculation.

EXPERIMENTAL RESULTS

The conclusions to be deduced from a consideration of mean values have been discussed above in conjunction with similar results by previous investigators. In many instances a scrutiny of the experimental figures will bear out the contention arrived at by comparing the mean values but in others the validity of a conclusion based on means is not so obvious.

More especially is this true where variation in experimental figures is great and the difference between non-pregnant and pregnant mean figures is small. It becomes of the utmost importance to prove whether such differences are significant or whether the random sampling of a larger number of pregnant and non-pregnant women would exhibit the same frequency distribution in each.

Stress has previously been laid on the fact that every factor known to affect the level of blood lipids except pregnancy has been eliminated in the present study. The age of patients did not affect these levels as would be expected from the relatively restricted age range in pregnant women. Parity was also found to have no influence. The analytical methods used were found dependable in the hands of the author (41). Under these conditions the difference between experimental values must be due to the biological variation of individuals which at present cannot be controlled.

The number of cases presented may appear limited. Subsequent analyses on pregnant and non-pregnant women have substantiated both the means and variations of the present figures. The "probable errors" in the means may be seen to be small, except in cases specifically mentioned below. Thus sufficient cases have been presented to demonstrate any difference between pregnant and non-pregnant women.

The experimental results have been reported in Tables I, II and III. In each column of figures the arithmetic mean and standard deviation have been calculated, the latter from the formula (42), $\theta = \sqrt{(\Sigma(x)^2)/n}$, where θ represents standard deviation, x the variation of each figure from the mean, n the total number of figures and Σ a summation symbol. From the mean and standard deviation it is possible to calculate the expected frequency distribution. Thus 68 per cent of cases will fall within the range, mean $\pm$ standard deviation, while 95 per cent of cases is contained within, mean $\pm 2 x$ standard deviation.

A concise idea of the blood lipid changes during pregnancy may be obtained by collecting the means and standard deviations of the four blood lipids, as in Table IV.

The results may be conveniently analysed in the reverse order in which they are tabulated here. The red blood cells showed no significant change

TABLE IV

Summary of mean values and standard deviations of observations

	Neutral fat	Phospholipid	Free cholesterol	Ester cholesterol
Whole blood, pregnant	248 ± 63	293 ± 52	84 ± 11	95 ± 29
Whole blood, non-pregnant	134 ± 54	256 ± 17	83 ± 76	83 ± 16
Plasma, pregnant	353 ± 75	248 ± 43	65 ± 15	140 ± 47
Plasma, non-pregnant	154 ± 77	195 ± 37	53 ± 10	128 ± 23
Red cells, pregnant	89 ± 80	361 ± 155	121 ± 43	16 ± 21
Red cells, non-pregnant	93 ± 42	361 ± 56	140 ± 26	6 ± 9

in their lipid composition during pregnancy. Occasionally small amounts of cholesterol ester were present both in the pregnant and non-pregnant. Considering the method of estimation, these figures may have been due to errors in technique. It is more than likely that there is very little if any cholesterol ester in the red blood cells of fasting women, pregnant or non-pregnant. The apparent discrepancy in Table III—that total cholesterol is less than the sum of free and ester cholesterol—is due to the fact that the method for total cholesterol determines 90 per cent of the lipid present while that for free cholesterol estimates 95 per cent.

In blood plasma neutral fat showed a prominent increase in pregnancy. From the values above it may be expected that approximately 75 per cent of pregnant women will have a higher plasma glycerol fat level than 75 per cent of non-pregnant women. The changes are less marked for the other lipids. Phospholipid and free cholesterol are each increased about one quarter on the average. Their frequency curves overlap considerably more than those for neutral fat. The mean value for ester cholesterol is barely 9 per cent higher in pregnancy and the frequency curve overlaps both extremities of that in the non-pregnant. As a result ester cholesterol averages 67 per cent of the total cholesterol as against 70 per cent in non-pregnant women. The ratio phospholipid/cholesterol, considered by many the most important lipid ratio in the blood, is 12 per cent higher in pregnancy, averaging 1.22 compared with 1.09 in normal women.

The lipemia of pregnancy is thus characterized by a marked increase in plasma neutral fat and smaller but appreciable increases in plasma phospholipid and free cholesterol. The lipid composition of the red blood cells is unchanged and the changes in whole blood are less marked. The latter need not be discussed further as no added information relative to the lipemia of pregnancy is thereby obtained.

In general the figures reported here are somewhat lower for all lipids than those recorded in the literature by the authors previously cited. This is no doubt in part due to the use of more exact methods for the isolation of lipids, distilled solvents and chemically clean apparatus, etc. But it should also be emphasized that the experiments herein contained were very carefully controlled. Not only was blood taken in the fasting state but the previous habitual diet of the patient was regulated, since habitual diet also affects the level of blood lipids. Muscular exercise, which may increase the neutral fat of blood, was minimized and all other known factors, as previously described (41), eliminated. It is felt, therefore, that these results, while they agree in nature with previous reports, indicate the more exact extent of lipemia due primarily to pregnancy. It has been found a real lipemia in most cases but less extensive than usually supposed. In no instance, may it be noted, was a " milky " plasma encountered. This old observation was probably made on the serum from uterine bleeding following the exhaustive first and second stages of labor. At this time it may

be calculated from Hellmuth's (33)'figures there occurs a tremendous increase in glycerol fat and this lipid in oily suspension, is largely responsible for the milky appearance of serum in lipemia.

The present report contains an innovation in studies on this subject in that it includes information as to the composition of plasma fatty acids. This was accomplished by micro-estimation of iodine numbers of the total and phospholipid fatty acids. Unfortunately, methods were not available for the micro-determination of iodine numbers of fatty acids from cholesterol ester and neutral fat. By referring to Table II it may be noted that the iodine numbers of both total and phospholipid fatty acids are very similar in pregnancy to those in non-gravid women (41). While on the average the iodine numbers of phospholipid fatty acids are 4 per cent lower in pregnancy, indicating more saturated fatty acids, the range was quite extensive and comparable to that in the non-pregnant. It may thus be concluded that the average composition of the fatty acids is similar in pregnancy to that in non-pregnant women. Since the phospholipid fatty acids exhibit the same degree of unsaturation in gestation as normal, it is more than likely there is no change in the composition of the fatty acids in cholesterol ester and neutral fat, although this remains to be proven experimentally.

DISCUSSION

Bloor (44) has divided lipemias into two types. The first or "temporary" lipemia occurs following ingestion of fat and need not concern us further. The lipemia of pregnancy appears to belong under the second or "persistent" group of lipemias although not mentioned by Bloor in his list of lipemias included here—diabetic, nephritic, alcoholic (chronic) and hemorrhagic. Characterizing the persistent group of lipemias especially hemorrhagic and diabetic lipemia, are the following changes in the lipids of blood. The lipemia is confined largely to blood plasma, the blood corpuscles showing relatively slight alteration. In blood plasma neutral fat increases first and most extensively, then "lecithin" and finally cholesterol, the latter usually exceeding the increase in "lecithin" as the height of the lipemia is reached. As a result the lecithin/cholesterol ratio becomes lowered at the height of the lipemia. As the lipemia disappears the lipids are lowered in the same order in which they were increased.

The lipemia of pregnancy is therefore very similar in nature to the lipemia produced in diabetes, nephritis, chronic alcoholism and persistent hemorrhage with the exception that in gestation the increase in phospholipid parallels that of cholesterol and the ratio, phospholipid/cholesterol, is little changed from its normal value of approximately unity. From the studies of Tyler and Underhill (19) and Hellmuth (33) throughout pregnancy, it may be calculated the earliest change in the blood lipids is an increase in neutral fat which occurs in the first trimester when the other lipids are

not raised. Phospholipid and cholesterol coincidently increase from the second trimester to term. The disappearance of pregnancy lipemia following parturition was similar in nature to that reported by Bloor (44) for other persistent lipemias and will be discussed in a subsequent paper.

Thus in pregnancy, diabetes, and continued hemorrhage occur lipemias of identical nature, the cause of which obviously cannot be the same in each case. Whatever the stimulus the initial response appears to be an increase in neutral fat which is followed by and probably instigates an increase in phospholipid and cholesterol. The increase in these latter two lipids appears entirely secondary to increase in neutral fat and probably represents some adjustment in the lipid balance of the body. On the other hand it might be said phospholipid and cholesterol respond later to the same stimulus which caused increase in neutral fat and that their increase is independent of the increase in neutral fat. If such were the case the widely different lipemia-stimuli in pregnancy, diabetes, hemorrhage, etc., must each produce exactly the same effect and in the same sequence. This does not seem likely. On the other hand it is quite possible that a simple increase in neutral fat might result from a wide variety of stimuli.

The *cause* of the lipemia of pregnancy must therefore be sought in the cause of the initial increase in neutral fat. No investigations have been directed at this particular angle of the question and, indeed, most of our ideas as to the etiology of the lipemia of pregnancy are hypothetical postulates unsupported by direct experimental investigation. There are about fifteen theories of this nature variously contained in the literature cited above (1, 4, 5, 6, 9, 10, 12, 14, 15, 19, 23, 33, 34, 35, 37) on fat metabolism during pregnancy.

The endocrine origin of the lipemia of pregnancy has been one of the most popular conceptions and has received favorable support by recent demonstrations that many hormones affect the level of blood lipids. Elden (45) has recently discussed the activity of the endocrine glands during the pre-mature, mature and post-mature sexual life of women including menstruation and the menopause. But there is no definite information as to the levels in blood or urine of the endocrine products during pregnancy and such evidence is the missing link in the application of the theory which relates the lipemia of pregnancy to the endocrine glands.

Other theories ascribe the origin of the lipemia of pregnancy variously to absorption of fat, chyle, milk, katabolic end products of fetal metabolism, retardation of the pulse, placental toxins, other toxins, remote effects of the fetus, decreased blood lipase, suprarenal synthesis, decreased lipid eliminations in bile, corpus luteum, increased cell destruction, immunological reaction, increased metabolic requirements for fats, lactation, etc. Many of these hypotheses seek to explain the increase of only one lipid, particularly the Bacmeister-Havers theory that cholesterol is increased in blood because of retarded elimination in bile through the liver. Since the hyper-

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cholesterolemia is probably a secondary reaction, such theories are inacceptable. None of the conceptions listed above have been experimentally demonstrated to explain the phenomenon and few have led to constructive knowledge of fat metabolism in pregnancy.

Whatever the cause of increased plasma lipids in pregnancy the *effect* of the lipemia on the maternal and fetal organism must be considered. Several physiological and pathological conditions are said to be due to the lipemia. Chauffard, Laroche and Grigaut (4) considered that increased cholesterol conferred a degree of immunity on the mother. Slemons and Stander (15) postulated that the increased fat of the blood provided a ready source of milk-fat following delivery. On the question of whether or not the placenta is permeable to lipids hinge the various statements (4, 6) that increase in maternal plasma lipid acts, as it were, as a greater pressure head forcing lipids into the fetal circulation where they are no doubt urgently required for the growing tissues of the embryo. But the permeability of the placenta to fats has been disputed since Ahlfeld's (1877) (47) demonstration that following a copious fat meal the maternal (dog) blood is milky while the fetal (pups) is clear. Sinclair (46) recently found the iodine numbers of fat fed the mother affected the iodine numbers of phospholipid and " fat " fatty acids of fetal rats and concludes the rat placenta is permeable to fats. Since pregnant rats do not exhibit an hypercholesterolemia as humans do, and probably also no lipemia, it is impossible to apply this evidence by analogy to pregnant women. Pathologically one or another lipid has from time to time been found increased, more than the usual for normal pregnancy, in the toxemias of pregnancy, pre-eclampsia and eclampsia. Slemons and Stander (15), reviewing the literature and presenting their own cases, conclude there is no significant variation in the blood lipids during the toxemias and eclampsia. Discussion of this phase of the subject will be reserved for a future publication.

CONCLUSIONS

Studies on the nature of the lipemia of pregnancy are incomplete and inconclusive unless the lipid content of whole blood, plasma and red blood corpuscles are simultaneously investigated, because the changes encountered are not the same in all three. In this fact lies the explanation of apparent discrepancies in the reports of previous workers who have investigated the blood lipids during pregnancy in women.

The lipemia of pregnancy is due almost entirely to increase in plasma lipids, the red cells showing but slight change and whole blood, as a result, giving little indication of what is taking place. In plasma, neutral fat begins to increase in the first trimester while phospholipid and cholesterol increase in the second trimeter. At term neutral fat is elevated the most (over 100 per cent) ; phospholipid and free cholesterol are each raised about one quarter over their value in non-pregnant women. The ratios of phos-

pholipid to cholesterol and ester cholesterol to total cholesterol are but slightly altered in the plasma of gravid women. These results place the lipemia of pregnancy in a group of similar persistent lipemias including diabetes and experimental anemias.

In spite of an increase in the amount of total and phospholipid fatty acids in blood plasma during pregnancy, there is no change in the composition of these fatty acids as evidenced by similar iodine numbers in gravid and non-gravid women.

The lipemia was found less extensive than reported by previous investigators, a fact which is due to the use of improved methods of lipid isolation and to control of the habitual diet of the patient; a "milky" plasma was not encountered.

The cause of lipemia of pregnancy appears to be the same as the cause of the early increase in plasma neutral fat. At present there is no adequate explanation for this.

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OBSERVATIONS ON THE CHEMICAL AND PHYSICAL RELATION BETWEEN BLOOD SERUM AND BODY FLUIDS. 1. THE NATURE OF EDEMA FLUIDS AND EVIDENCE REGARDING THE MECHANISM OF EDEMA FORMATION [1]

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An understanding of the derangements in the volume and the chemical composition of the body fluids in diseases associated with edema is essential to rational treatment. Comparative chemical measurements of the constituents of edema fluids and blood serum give insight into the nature of edema fluids, and consequently increase the knowledge concerning the mechanisms involved in controlling the volume and composition of these body fluids.

In the present investigation, the distribution of certain electrolytes, non-electrolytes and protein between serum and chest, ascitic, and subcutaneous edema fluids in various pathological conditions has been studied. The relative changes in the concentration of the constituents of the transudates and sera following the administration of salyrgan, ammonium chloride and sodium bicarbonate have been observed. The data have been analyzed to discover how closely the composition of edema fluids corresponds to the predicted chemical composition of simple dialysates of blood plasma.

METHODS

All samples of blood and body fluids were drawn after a fast of at least twelve hours, the chest, ascitic or subcutaneous edema fluids being taken either just before or just after withdrawal of the blood. The freezing point determinations reported by Loeb et al. (1) for chest fluid, ascitic fluid and blood serum, by Fremont-Smith et al. (2) for spinal fluid and blood serum, and by Gollwitzer-Meier (3) for subcutaneous edema fluid and blood serum, show similar depressions of freezing point for the fluids and sera when drawn after a fast similar to that observed in our experiments. None of the patients studied had a history of recent vomiting, diarrhea, or profuse perspiration. Venous blood was drawn without stasis from the antecubital vein, and arterial blood from the radial artery. The blood, chest fluid, and ascitic fluid were received anaerobically into oiled syringes and transferred under oil to centrifuge tubes. no anticoagulant being used. One-holed rubber stoppers were inserted into the

small oil layers at the tops of the tubes, and, after oil had been displaced through the hole, a glass plug was inserted tightly to exclude all the air. The blood samples were centrifuged as soon as possible after removal, the corpuscles being thrown down usually before the blood clotted. The clot was gently broken with a fine glass rod, the blood recentrifuged, and the serum removed immediately. A portion was collected over mercury for the determination of carbon dioxide content. The samples of chest and ascitic fluid were also centrifuged immediately after withdrawal and the fluid removed, a portion being collected over mercury. (There was sometimes a small pad of red cells in the bottom of the tube after the chest and ascitic fluids had been centrifuged.) Subcutaneous edema fluid was obtained by means of Southey tubes which were inserted beneath the skin of the lower extremity, usually in the dorsum of the foot, and were connected with four inch rubber tubes of 1 mm. bore leading into small test tubes containing oil. None of the samples studied contained blood. The samples were stoppered in the manner described above. Carbon dioxide content was determined immediately after collection.

The following chemical methods were used: chloride, Wilson and Ball (4); sodium, Rourke (5), or Butler and Tuthill (6) (identical results were obtained by these two methods); carbon dioxide content, Van Slyke and Neill (7); specific gravity (bottles containing 5 cc. were used), Moore and Van Slyke (8); protein of chest and ascitic fluid and of serum, Dyer (9) (nonprotein nitrogen was subtracted from total nitrogen and the remaining nitrogen multiplied by the factor 6.25); protein of subcutaneous edema fluid by Dyer (9), or Denis and Ayer (10), using precipitated protein standards; potassium, Fiske and Litarczek (11); creatinine, Folin (12); sugar, Folin and Wu (13); nonprotein nitrogen, Folin and Wu (14); inorganic phosphate, Fiske and Subbarow (15); calcium, Fiske and Logan (16); albumin and globulin contents of the fluids, Wu (17); and water content, by drying a weighed sample of approximately 2 grams at 105° C. for 48 hours. In a few experiments where the water content was not measured, it has been calculated from the formula: water in grams per 100 cc. $= 99.6 - 0.85$ P (P $=$ grams of protein per 100 cc. of fluid). We found that this formula agreed well with the determined values for all types of body fluids, including serum. From the total carbon dioxide content of serum and fluids one twenty-first is subtracted, the remainder being assumed to represent bicarbonate. To convert inorganic phosphorus into milli-equivalents it has been assumed that the valence of phosphorus is 1.8 at the approximate reaction of the body fluids, pH 7.4.

RESULTS AND DISCUSSION

Twenty-seven studies have been made in seventeen patients with congestive heart failure, nephritis with edema, portal cirrhosis with ascites and carcinoma with ascites. In nine instances, subcutaneous edema fluid, chest fluid and blood serum were obtained simultaneously. In one instance, subcutaneous edema fluid, ascitic fluid and blood serum were obtained simultaneously. The concentrations of electrolytes, of protein, sugar, nonprotein nitrogen and creatinine have been measured. Ten studies have been made to determine whether the administration of ammonium chloride, sodium bicarbonate and salyrgan affect the concentrations of electrolytes in edema fluids and serum to an equivalent extent.

In some studies venous blood serum was employed; in others, both arterial and venous blood sera were analyzed; in the majority of experiments, arterial blood serum alone was employed.   For many of the constituents studied the concentrations in arterial and venous blood sera are so nearly identical that, for the purpose of this study, it would be immaterial which sample was employed.   The arteriovenous differences in bicarbonate and chloride, however, are appreciable, and the source of the blood sample, especially in reference to the bicarbonate, is important.   Although absolute physicochemical equilibrium is not obtained under physiological conditions, plasma filtrates in the body are probably in approximate equilibrium with plasma of a character intermediate between that of the arterial blood entering and the venous blood leaving the capillaries involved.   The exclusive use of either venous or arterial blood may lead, therefore, to erroneous conclusions.   Since venous blood from different areas of the body varies considerably in composition, comparative studies of the equilibrium between venous blood from the arm and of fluid from the thoracic or peritoneal cavities or from the subcutaneous tissue spaces of the legs involve certain erroneous assumptions.   Arterial blood is at least uniform throughout the body, and therefore offers a constant and more satisfactory basis of comparison.   All the studies reported in the literature concerning the distribution of substances between serum and edema fluids in man have been made on the basis of venous blood from the arm.   It is interesting, in this connection, that the concentration of bicarbonate in the venous serum from the arm has generally been found to be higher than the concentration of bicarbonate in edema fluid, a distribution which is contrary to the demands of Donnan's equilibrium.   For this reason, it might be postulated that a fraction of the bicarbonate of the serum is " bound."   Comparison of the bicarbonate content of edema fluids with that of both arterial and venous sera should yield evidence concerning the state of the serum bicarbonate.

I.   Distribution of electrolytes between blood serum and ascitic, chest and subcutaneous edema fluid

The concentrations of electrolytes in the sera and body fluids are estimated in milli-equivalents per 1000 grams of water (Table I).   The ratios of the concentrations per 1000 grams of water of the ionized substances in the sera and corresponding transudates have been calculated, and the average ratio between sera and all transudates is given for the purpose of simplifying discussion (Table II).   The consideration of these edema fluids as a single group is justifiable since the data show that, when two fluids with approximately the same protein contents are drawn simultaneously from two different sources such as the chest and subcutaneous spaces, the values for the concentrations of all the electrolytes in the two fluids are practically identical.   The average ratios for bicarbonate and chloride are

TABLE I

The concentrations of electrolytes and of protein in serum and transudates

Units — Specific gravity: (ratio); Water: grams per 100 cc.; Protein: grams per 100 grams H₂O; Chloride, Bicarbonate, Phosphate, Sodium, Potassium, Calcium: m. Eq. per 1000 grams H₂O.

Case no.	Diag.*	Day	Medication	Blood†	SG Serum	SG Chest	SG Ascitic	SG Subcut.	H₂O Serum	H₂O Chest	H₂O Ascitic	H₂O Subcut.	Prot. Serum	Prot. Chest	Prot. Ascitic	Prot. Subcut.	Cl Serum	Cl Chest	Cl Ascitic	Cl Subcut.	HCO₃ Serum	HCO₃ Chest	HCO₃ Ascitic	HCO₃ Subcut.	PO₄ Serum	PO₄ Chest	PO₄ Ascitic	PO₄ Subcut.	Na Serum	Na Chest	Na Ascitic	Na Subcut.	K Serum	K Chest	K Ascitic	K Subcut.	Ca Serum	Ca Chest	Ca Ascitic	Ca Subcut.
1	N	1		V	1.0216	1.0096		1.0085	95.4‡	99.1‡		99.4‡	5.20	0.50		0.29	123.1	126.1		126.7	21.0	20.2		20.1	4.2	4.0		4.3	154.1	148.4		143.5		4.7		4.8	4.4	4.3		3.2
1	N	23	NaHCO₃	V	1.0204	1.0102		1.0089	95.8‡	98.9‡		99.4‡	4.72	0.83		0.26	97.8	102.5		102.0	38.5	37.3		36.8	4.1	4.0		3.9	152.6	147.4		147.6		3.5		3.5	3.6	2.6		2.7
2	C, D	1		V	1.0253	1.0128			94.1	98.0			6.95	1.90			105.8	107.6											149.6	142.8			5.5	4.2			4.7	3.7		
3	N	1		V	1.0196	1.0112		1.0093	95.6‡	97.9‡		98.7	4.09	2.09		1.11	120.2	123.6		120.6	20.0				3.7	3.6			136.0	137.3		131.4					4.7	3.3		3.4
3	N	12	NaHCO₃	V	1.0205	1.0119			95.1‡	97.8‡		98.6	5.51	2.19		1.19	104.7	106.2		105.3	33.2								144.1	139.6		138.3					4.5			
3	N	16		V	1.0220				95.0			98.5	5.76			1.32	105.2			106.0	33.3								143.5			136.3								
4	C	1		V	1.0248	1.0130	1.0126		94.5‡	97.9‡	97.8‡		6.37	2.01	2.15		118.2	109.5	119.0							2.3			148.6		140.9									
5	C	1		V				1.0078				99.2‡				0.47	106.8			120.9	29.8				2.4				141.8			140.3	6.0				4.5			
6	C, D	1		A	1.0260	1.0130			93.8	97.7		98.9	7.35	2.12		0.86	110.7	112.0		112.4	26.5	29.1		20.8	2.6	2.5		2.4	144.7	142.1		139.9		5.2		4.2	5.0	3.2		3.0
6	C, D	10	NH₄Cl	A	1.0261	1.0144			93.7	97.6		98.8	7.35	2.45		0.91	119.8	121.2		121.5	19.9	22.7			2.9	2.7			147.6	144.5		142.4		4.8			4.9	3.0		
6	C, D	11	Salyrgan	A	1.0263	1.0151			93.7	97.4		98.8	7.42	2.62		0.91	120.2	120.9		120.7	20.0	21.8			2.9	2.8			147.7	143.4				5.0			4.6	3.8		
7	C	1		A	1.0234	1.0109			94.3	98.8		99.3	6.54	1.22		0.60	109.4	110.6		111.9	28.7	31.9			2.1	2.3			146.1	145.2		143.6					4.7	3.4		
7	C	2	Salyrgan	A		1.0120			94.8	98.4		99.3	6.14	1.57		0.60	110.8	110.8		112.8	33.3	35.4							151.3	148.5		146.3						3.5		
8	C	1		A	1.0228			1.0090	94.8			99.3	5.66			0.25	89.6			92.1	30.8			33.4	3.8			3.6	143.6			136.7				5.0	4.8			3.4
9	Ci	1		A	1.0239		1.0110		94.9		98.4		5.86		1.42		94.4		97.4		25.4		27.7		2.4		2.2		134.1		127.6				5.3		4.6			
10	C	1		A	1.0274				93.4			99.2	8.12			0.50	113.7			120.9	19.5			23.5					147.7			140.4				5.0				
11	C	1		A	1.0256	1.0143			93.7	97.6			7.30	2.55			107.6	109.4			28.2	31.0			2.7	2.6			150.7	145.1			5.6	4.5			5.3	3.9		
11	C	2	Salyrgan	A	1.0254	1.0150			93.4	97.6			7.22	2.76			106.6	108.2			28.2	31.9			2.8	2.8			151.4	146.4			6.1	4.4			5.3	4.0		
12	C, D	1		V	1.0258	1.0126			93.9	98.5			7.17	1.79			108.8	110.0			28.1	31.5			2.0	1.9			155.6	147.0			5.1	4.3			5.3	4.0		
12	C, D	2	Salyrgan	A		1.0136			93.9‡	98.1			7.14	2.11			106.4	107.9			31.2	33.9							155.2	147.6				4.2				4.0		
13	Ca	1		A	1.0253		1.0182		94.3		96.2		6.75		4.36		106.8		108.5		25.6		27.2		2.0		2.0		151.8		150.0						4.9		3.5	
14	Ca	1		A	1.0239		1.0130		94.4		98.0		6.47		2.09		107.3		109.7		25.2		26.9		1.9		1.9		148.9		141.9		4.8		4.4		4.6		4.3	
15	Ca	1		A	1.0258		1.0167		94.1		96.7		7.18		3.65		107.4		109.1		28.3		31.0		2.1		1.8		146.5		142.5								3.9	
16	C, D	+3		V	1.0255	1.0103			94.1	99.0		99.4	7.02	1.08		0.60	111.7	113.6		117.3	23.2	25.1		30.2					149.6	140.6		148.0	5.8							
16	C, D	13		A	1.0252	1.0103			94.0	99.0		99.4	6.98	1.08		0.60	112.5	113.6		117.3	21.9	25.1		30.2					147.9	140.6		148.0	6.1							
17	C, D	1		V	1.0232				94.3				6.08				112.6				30.3								155.0											
17	C, D	1		A	1.0226				91.6				5.94				113.8				28.5								154.0											

* N, nephritis; C, cardiac decompensation; D, diabetes; Ci, cirrhosis of the liver; Ca, carcinoma.

† V, venous blood; A, arterial blood.

‡ Water content calculated from protein content.

TABLE II

Concentration ratios of electrolytes in serum and transudates

Case number	Diagnosis	Day of study	Medication	Blood sample	Chloride Serum/Chest fluid	Chloride Serum/Ascitic fluid	Chloride Edema fluid/Serum	Bicarbonate‡ Serum/Chest fluid	Bicarbonate‡ Serum/Ascitic fluid	Bicarbonate‡ Edema fluid/Serum	Phosphate‡ Serum/Chest fluid	Phosphate‡ Serum/Ascitic fluid	Phosphate‡ Edema fluid/Serum	Sodium Serum/Chest fluid	Sodium Serum/Ascitic fluid	Sodium Edema fluid/Serum	Potassium‡ Serum/Chest fluid	Potassium‡ Serum/Ascitic fluid	Potassium‡ Edema fluid/Serum	Calcium‡ Serum/Chest fluid	Calcium‡ Serum/Ascitic fluid	Calcium‡ Edema fluid/Serum
1	N*	1	NaHCO₃	V†	0.974		0.972	1.040		1.046	1.055		0.972	0.962		0.963				0.744		0.719
1	N	23		V	0.954		0.959	1.031		1.045	1.033		1.035	0.966		0.968				0.731		0.756
2	C, D	1	NaHCO₃	V	0.983						1.025			0.954			0.769			0.795		0.752
3	N	12	NaHCO₃	V	0.972		0.997			0.954	1.035			1.009		0.966						0.713
3	N	16		V	0.986		0.995							0.969		0.959						
4	C	1	NH₄Cl Salyrgan	V	0.976		0.992	0.989			1.035			0.965		0.950	0.866					0.655
5	C	1	Salyrgan	V		0.969	0.978		0.918			1.063	0.891		0.952	0.941		0.892			0.758	0.713
6	C, D	10		A	0.988		0.986	0.911			1.015			0.983		0.966	0.864		0.831	0.700		
6	C, D	11		A	0.988		0.988	0.875			1.063			0.979		0.964	0.896		0.844	0.722		
6				A	0.991		0.996	0.921			1.010			0.972						0.781		
7	C	1	Salyrgan	A	0.989		0.978	0.900			0.930		1.033	0.996		0.984	0.830		0.844	0.766		
7		2		A	1.000		0.982	0.939						0.982		0.968				0.736		
8	C	1		A		0.985	0.973		0.912	0.920		1.020			0.988	0.953		0.919			0.844	
9	Cl	1		A		0.978	0.911		0.935	0.832		1.005			0.953	0.951					0.852	
10	C	1		A		0.981			0.914			1.125			0.972							
11	C, D	1	Salyrgan	A	0.984			0.908			1.012			0.963			0.772			0.738		
11		2		A	0.986			0.881			1.021			0.967			0.710			0.745		
12	C, D	1	Salyrgan	A	0.989			0.802			1.080			0.944			0.844			0.743		
12		2		A	0.986			0.920						0.952								
13	Ca	1		A																		
14	Ca	1		A																		
15	Ca	1		A																		
16	C, D	13		V	0.983		0.960	0.926		1.005				1.010		0.955						
16	C, D	13		A	0.990		0.970	0.872		0.917				0.952		0.961						
17	C, D	1		V / A																		
Average ratio $\dfrac{\text{Venous serum}}{\text{Fluid}}$					0.978 ± .002			1.012 ± .011			1.026 ± .006			0.961 ± .002			0.837 ± .011			0.748 ± .006		
Average ratio $\dfrac{\text{Arterial serum}}{\text{Fluid}}$					0.983 ± .002			0.910 ± .005														
Average ratio $\dfrac{\text{Serum (Arterial and venous)}}{\text{Fluid}}$																						

* and † signify same as in Table I.
† These ratios have been calculated from the concentrations expressed to the second decimal place, the values in Table I being rounded off to the first decimal place.
§ Probable error of mean.

given for both arterial and venous bloods; for the other constituents no differentiation is made.

The concentrations per 1000 grams of water, of the *bases* sodium, potassium, and calcium, are uniformly *higher* in the *sera* than in the corresponding body fluids, while the concentrations of the *anions*, chloride and bicarbonate, are uniformly *lower* in the *arterial sera* than in the corresponding body fluids (Table I).

Regardless of the nature of the fluid, whether chest, ascitic, or subcutaneous edema fluid, there is an approximate straight line relationship for the differences in protein concentration and differences in chloride concentration between the sera and fluids (Figure 1). There are two factors
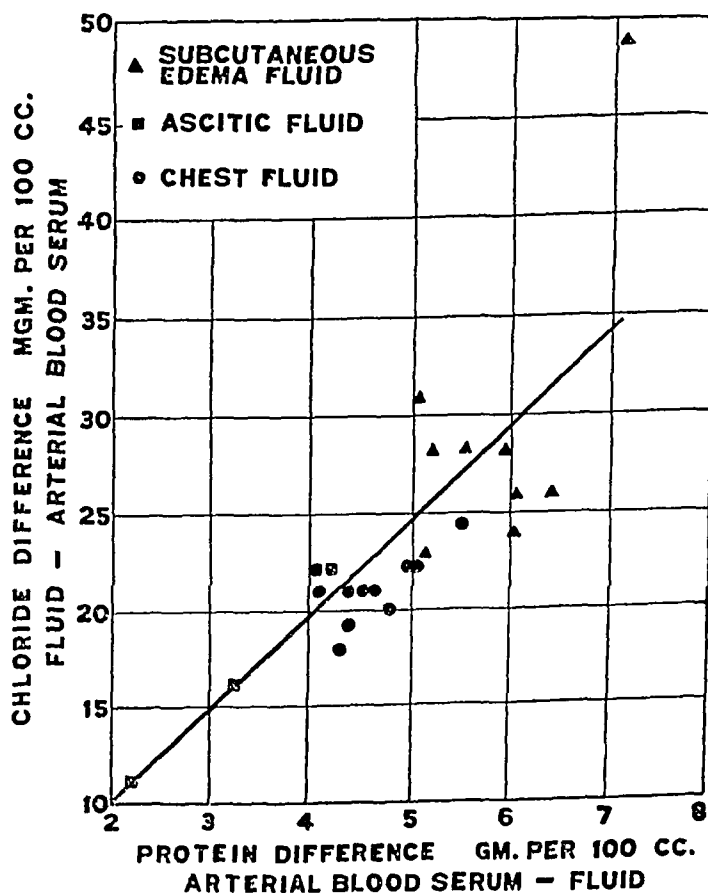
FIG. 1

The difference in concentration of the protein of the arterial serum and corresponding body fluid (grams serum protein per 100 cc. minus grams fluid protein per 100 cc.) is plotted against the difference in the concentration of the chloride of the fluid and serum (mgm. fluid chloride per 100 cc. minus serum chloride per 100 cc.).

which determine this relation in Figure 1: the relative water contents of the fluids and sera, and the relative distribution of the ions on the basis of the water contents of the fluid and sera. The same type of linear relation-

ship holds also in a rougher way for the differences in bicarbonate and protein concentration between arterial sera and corresponding fluids.

The application of the Donnan theory of membrane equilibrium to the distribution of electrolytes between serum and edema fluids. According to this theory, the distribution of the ions between the serum and corresponding fluid is expressed mathematically by the equation:

$$r = \frac{(CL) \text{ serum}}{(CL) \text{ fluid}} = \frac{(HCO_3) \text{ serum}}{(HCO_3) \text{ fluid}} = \frac{(Na) \text{ fluid}}{(Na) \text{ serum}} = \frac{(K) \text{ fluid}}{(K) \text{ serum}}, \text{ etc.,}$$

where the concentrations refer to the "activities" of the various ions. Accurate quantitative values for the "activities" of the various ions in a system as complicated as that comprising blood plasma and body fluid, are not available. As a first approximation, and in accord with the general practice of others in studies such as these, the theoretical Donnan ratio has been calculated on the basis of the concentrations of inorganic anions per kilo of serum H_2O; in our cases in serum representing an average of arterial and venous blood. The formula derived by Van Slyke, Wu and McLean (18) for the Donnan distribution ratio between serum and transudates has been employed, the base bound by protein being estimated with the aid of the formulae of Peters, Wakeman, Eisenman and Lee (19), assuming a pH of 7.35 for serum and fluids. This approximate mathematical expression of the Donnan ratio is used below more to discover whether our results conform in general to the dictates of the Donnan theory of equilibrium than with the expectation of obtaining absolute mathematical conformity. For our measurements the average Donnan ratio, estimated in this manner, is 0.955. The distribution ratios found in our studies (Table II) are also compared to the ratios found by Greene and Power (20) for blood plasma and experimental dialysates in dogs, these dialysates being obtained by an ingenious method of compensation dialysis " in vivo."

The average ratio, (CL) serum/(CL) edema fluid, found in our studies was 0.978 when venous serum was employed, and 0.983 when arterial serum was employed (Table II). The average ratio, (Na) edema fluid/ (Na) serum, was 0.96.

The average ratio, (HCO_2) serum/(HCO_3) edema fluid, was 1.012 for the venous serum studies, and 0.910 for the arterial serum studies. The high average ratio, (HCO_2) serum/(HCO_2) fluid, of 1.012 for the venous serum studies is in accord with the bicarbonate ratios found by other investigators (3) (22) (23), contrasted with a ratio of less than unity to be expected at equilibrium according to the Donnan theory. The wide difference between the bicarbonate ratio found in the series where arterial blood was employed, and that found when venous blood was used (Table II), demonstrates the importance of considering the source of blood samples in studies such as these. Since the ratio, (HCO_3) serum/(HCO_2) fluid, was 0.910 for our arterial serum studies, it is unnecessary to postulate " bound "

bicarbonate.   Darrow, Hopper and Cary (21) in four instances compared the concentration of bicarbonate in ascitic fluid from dogs rendered edematous by lowering the plasma protein concentration by plasmapheresis, with the concentration of bicarbonate in blood from both femoral artery and jugular vein.   These authors likewise found the ratio, (HCO_3) serum/(HCO_3) ascitic fluid, to be greater than the theoretical Donnan ratio when venous blood was employed and less than the theoretical ratio when arterial blood was employed.   Further, the average ratio, (HCO_3) serum/(HCO_3) dialysate, found by Greene and Power (20) in their studies on " in vivo " dialysates in dogs was 0.97 as compared to the ratio, (Cl) serum/(Cl) dialysate, 0.98.   In Greene's studies, the blood sample representative of equilibrium conditions was known.

The mean ratio for our studies, (HCO_3) serum/(HCO_2) fluid, representative of blood midway between arterial and venous, was 0.96.   The approximate mathematical conformity of this ratio, with the ratio, (Cl) serum/(Cl) fluid, 0.98, the ratio, (Na) fluid/(Na) serum, 0.96, and the theoretical ratio 0.955, suggests that the edema fluids studied are in equilibrium with blood plasma having a bicarbonate concentration midway between that of an arterial and that of a venous sample and that none of the bicarbonate of the serum exists in non-diffusible form.

The distribution of calcium, potassium, and inorganic phosphate between serum and the transudates studied confirms the results of other investigators (1) (3) (22) (23) (24), and indicates that a part of these substances exists in the serum in a non-diffusible form.   The Donnan membrane effect can explain only in small part the fact that the concentrations of potassium and of calcium are considerably lower in the transudates than in the sera (Table I).   The results obtained for calcium are to be expected, since it is well known that calcium is present in serum in more than one form (25) (26), part of the calcium being non-diffusible, probably in combination with protein, as experiments with collodion membranes have shown.   The average inorganic phosphate concentration of the serum was slightly higher than that of the fluids.   These results confirm those of Greene et al. (20) (23) and indicate that a very small fraction of the inorganic phosphate may be held in the serum in non-diffusible combination.

Since the results for the distribution of the electrolytes between serum and chest, ascitic, and subcutaneous edema fluids are, on the basis of our present knowledge, in close harmony with Donnan's theory and with the results obtained by Greene et al. in studies of " in vivo " dialysates, it may be concluded that these transudates, insofar as electrolyte concentrations are concerned, conform to the expected composition of simple dialysates.

II.   Distribution of non-electrolytes between blood serum and ascitic, chest and subcutaneous edema fluids

Available data dealing with the distribution of non-electrolytes between serum and chest, ascitic and subcutaneous edema fluids are meager.   In our

studies the average concentration of total reducing substances (sugar, etc.) per 100 grams of water (Table III) in the blood serum was found to be somewhat lower than the average concentration in the corresponding chest, ascitic, and subcutaneous edema fluids. No distinction has been made be-

TABLE III

Concentrations of non-electrolytes in serum and transudates

Case number	Diagnosis	Sugar				Nonprotein nitrogen				Creatinine			
		Serum	Chest fluid	Ascitic fluid	Subcutaneous edema fluid	Serum	Chest fluid	Ascitic fluid	Subcutaneous edema fluid	Serum	Chest fluid	Ascitic fluid	Subcutaneous edema fluid
		mgm. per 100 grams H_2O				*mgm. per 100 grams H_2O*				*mgm. per 100 grams H_2O*			
1	N*					44	45		42	1.9	2.0		1.9
1	N					37	38		33	1.8	1.8		1.7
2	C, D					43	43						
3	N					78	78			2.3	2.2		
3	N					35	37			1.5	1.3		1.3
5	C	111	118			36	34			1.5	1.4		
6	C, D	184	194			43	40						
6	C, D	157	184		200	53	54		51				
6	C, D	169	185			57	65						
7	C	103	105			30	29						
7	C	92	100			32	31						
8	C	123			135	129			131				
9	Ci	119		145		41		41					
11	C	93	102			40	38						
11	C	103	105			50	46						
12	C, D	161	164			44	39			1.5	1.4		
13	Ca	99		84		30		31		1.4		1.4	
14	Ca	111		113		33		32		1.2		1.2	
15	Ca	92		110		34		35					
16	C, D	173	202			36	33						
17	C, D	147			146	26			25				
18	Ci, D	182		181		25		25		1.3		1.3	
19	Ci	130		129		27		29				1.3	
Average ratio $\dfrac{\text{All fluids}}{\text{Serum}}$		1.06 ± .015 †				0.98 ± .008				0.97 ± .017			

* Letters refer to diagnoses as given at bottom of Table I.
† Probable error of mean.

tween the concentrations in arterial and venous sera since the arteriovenous difference in fasting blood sugars is negligible (27).

Power and Greene (28) have shown by experiments on the "in vivo" dialysate of circulating arterial blood in dogs that, at equilibrium, the concentration of glucose in the dialysate is practically equal to that in the plasma, wherefore they conclude that the plasma glucose is freely diffusible

and that there is no non-diffusible or colloid bound sugar in the plasma. In seven of our cases, the differences in concentrations of reducing substances in the transudates and in the corresponding sera did not exceed experimental errors. In view of the many extraneous factors which influence the fasting sugar level, the differences in the concentrations found in the remaining cases may be due to absence of equilibrium conditions. The greatest differences were observed in diabetic cases, where the sugar of transudates was higher than that of the serum. This may be referable to the greater instability of the blood sugar in diabetes; the fluid sugar having not yet come into complete equilibrium with the serum sugar at the time of collection of the samples. It should be noted that apparent lack of equilibrium for sugar in these cases does not invalidate the conclusion that the specimens are in approximate equilibrium as regards other constituents. No other constituent of the body fluids which was measured has a variation comparable to sugar, even in normal individuals. The equal distribution of reducing substances between transudates and corresponding sera found in seven of the above studies indicates, in accord with Power and Greene's studies, that the glucose of the human plasma is freely diffusible. The results indicate that under equilibrium conditions glucose is distributed in accord with the concept that the fluids studied are simple dialysates of serum.

The concentrations of creatinine and total nonprotein nitrogen in ascitic, chest and subcutaneous edema fluids were essentially the same as the concentrations of these substances in the corresponding sera when the values were expressed in relation to units of water (Table III). This finding is in accord with what would be expected for the distribution of non-electrolytes between two fluids separated by a membrane permeable to these substances.

III. Protein contents of serum and transudates

The fluid protein varied from 0.25 gram per 100 grams of fluid water for the subcutaneous edema fluid in a case of cardiac edema to 4.36 grams in a case of ascites secondary to carcinoma (Case 13); the average value for all the fluid proteins was 1.49 grams per 100 grams of water (Table I). In general, the lowest protein values were found in the subcutaneous edema fluids, both nephritic and cardiac, and the highest values were found in the ascitic fluids in patients with carcinoma. Beyond this generalization, it is impossible to anticipate the protein content of a given fluid in cases as reported above. The concentrations of total protein in edema fluids from patients with nephritis may be the same as those from patients with congestive heart failure. Rate of formation, rate of reabsorption (both capillary and lymphatic), and capillary permeability all play a part in determining the protein contents of edema fluid.

The effect of salyrgan diuresis on the concentration of protein in chest fluid was studied on four occasions (Table I, Cases 6, 7, 11, 12). A decrease in the volume of fluid and a significant increase in the protein content of the fluid were observed in each instance. This finding, which has been reported previously by Iversen and Johansen (29), indicates a more rapid reabsorption of water and salts than of protein from the edematous deposits during salyrgan diuresis. We have also observed (39) a marked increase in the protein concentration of the chest fluids and subcutaneous edema fluids of cardiac patients during diuresis caused by digitalis and rest in bed.

The ratios of the amounts of albumin to the amounts of globulin in the various body fluids varied considerably (Table IV). The same variable factors that determine the total protein contents of edema fluids must also determine the relative proportions of albumin and globulin.

TABLE IV
Albumin and globulin of chest and ascitic fluids

Case number	Diagnosis	Fluid	Albumin	Globulin
			grams per cent	grams per cent
6	C, D*	Chest	1.79	0.68
16	C, D	Chest	0.58	0.31
12	C, D	Chest	1.26	0.25
12	C, D	Chest	1.41	0.45
9	Ci	Ascitic	1.02	0.33
13	Ca	Ascitic	2.86	1.49
14	Ca	Ascitic	1.51	0.50
19	Ci	Ascitic	0.77	0.57
19	Ci	Ascitic	0.75	0.41

* Letters refer to diagnoses as given at bottom of Table I.

A tendency toward moderate reduction of serum protein and specific gravity in cases with edema of diverse origin is evident from our results (Table I). The serum protein for all cases averaged 6.50 grams per 100 grams of serum water and the specific gravity averaged 1.0241; both the serum protein values below 5 grams were encountered in the two cases of nephritis (Cases 1 and 4). That the serum protein is low as a rule in cases of nephritis with edema has been shown graphically by Moore and Van Slyke (8). The protein reduction is referable mainly to decreases in the albumin fraction (8), and the depletion is due to albumin losses in the urine, together with dietary deficiencies of protein in some instances (30). Albuminuria as a result of co-existent congestive heart failure, rapid loss of serum protein into the edematous deposits of the chest and peritoneal cavities with repeated removal and reaccumulation, and malnutrition (31), all probably play a rôle in the moderate reduction of serum proteins

in the non-nephritic cases reported above. Payne and Peters (31) and Muntwyler et al. (32) have likewise shown that the plasma protein is sometimes low in cardiac patients with edema. It is unfortunate that patients with congestive heart failure and increased venous pressure should so frequently have low serum proteins, to dispose them still further toward the formation of edema. It is possible that the low protein content found in some cardiac patients may, in the presence of mild venous congestion, be the decisive factor in edema formation.

IV. The relative distribution of electrolytes between serum and transudates following sodium bicarbonate, ammonium chloride and salyrgan diuresis

The effect of the oral administration of sodium bicarbonate on the electrolytes of the blood serum, chest, and subcutaneous edema fluids was studied in two cases of nephritis acidosis with edema (Cases 1 and 3) (Table I). Before bicarbonate therapy, abnormally high serum and fluid chloride concentrations and abnormally low serum and fluid bicarbonate concentrations were found in these cases. In Case 3 the sodium of the serum and fluids was moderately decreased. Increases of both serum and fluid bicarbonate and decreases of serum and fluid chloride occurred in both cases following the ingestion of large doses of bicarbonate. In Case 3 the concentrations of sodium both in the serum and fluids increased to normal (Table I). The distribution ratios of these constituents, therefore, were not significantly altered (Table III).

Oral administration of ammonium chloride to a patient with edema of cardiac origin (Case 6, Table I) caused a marked increase in the chloride and decrease in the bicarbonate concentrations of the serum and fluids, the distribution ratios for these substances remaining unaltered.

The effect of salyrgan diuresis on the acid-base equilibrium of the blood serum, chest, and subcutaneous edema fluids was measured in four cases with cardiac edema (Table I, Cases 6, 7, 11 and 12). The "net" twenty-four hour diuretic effect was measured by subtracting the average daily urine output while the patient was on a constant fluid intake from the urinary output on the day of diuresis. Two cc. of salyrgan were given intravenously on each occasion at 9:00 A.M. after fasting samples of blood and fluids had been obtained. On the following morning at 9:00 A.M. samples of blood and fluids were again taken.

No appreciable changes in the electrolyte concentrations of the serum or edema fluids were found following 2 cc. of salyrgan in Cases 6 and 11 (Table I); the twenty-four hour diureses in these two patients were 2500 cc. and 1000 cc. respectively. We have likewise found no appreciable alterations in the serum of normal subjects following salyrgan. In Case 7, when the diuresis was 5500 cc., the bicarbonate and sodium concentrations of both serum and edema fluids increased appreciably. In Case 12 when

the diuresis was 1300 cc. the chloride concentrations of the serum and of the chest fluid decreased appreciably and the bicarbonate concentrations of the serum and fluid increased correspondingly.

In all instances, when serum electrolyte disturbances occurred in nephritis, or following medication, compensatory changes were found in the edema fluids so that the ratios of the concentrations of the constituents of edema fluids to the concentrations of the constituents of serum remained normal (Table II). *These findings demonstrate further that edema fluids have the characteristics of simple dialysates of blood plasma.*

COMMENT

The laws of chemical equilibrium which determine the relative concentrations of electrolytes and non-electrolytes in the serum and chest, ascitic and subcutaneous edema fluids studied, appear to be the same, regardless of the site of fluid formation or the underlying pathological condition.

The data accumulated show that these chest, ascitic and subcutaneous edema fluids have the nature of simple dialysates. These results are in agreement with the concept of McLean (34), that edema is a quantitative rather than a qualitative deviation from normal. From this it would follow that the fluids above studied accumulate in abnormal amounts in the body, primarily by virtue of a quantitative imbalance of the normal factors which control the formation and reabsorption of the interstitial fluids according to the Starling theory; namely, colloid osmotic pressure of the plasma, capillary pressure, tissue pressure and capillary permeability.

The fact that the concentration of proteins in subcutaneous edema fluid, both from patients with nephritis and from patients with cardiac decompensation, may at times be as low as 0.2 to 0.3 gram per 100 grams of water (Table I) is not in harmony with the concept that increased capillary permeability is the primary cause of edema in these instances.

The high incidence of edema in nephritic patients with plasma protein concentrations less than 5.5 grams per 100 cc. has been pointed out by Moore and Van Slyke (8). The lowered osmotic pressure in these cases is considered the primary cause of edema. McClure (35) has recently shown that edema of the nephrotic type may disappear in certain cases without appreciable increases in the lowered plasma protein and considered that changes in the tissues may play an important rôle in the production and dissipation of edema. The bulk of the evidence at present available in the literature shows that edema does occur at some time or another in nephritic patients with low plasma proteins and that the low plasma protein concentration is probably the primary cause of the edema. That the edema may disappear in certain instances without appreciable increase in the lowered plasma protein content may be due to changes, under the usual medical treatment, in other factors which control the volume of the interstitial body fluids, such as restriction of sodium chloride or a slight

decrease in venous pressure. These factors may, at a given critical level, be sufficient to turn the balance toward the dissipation of, rather than the formation of edema.

That edema fluid is not retained without sodium chloride is shown by the relative constancy of the content of sodium and chloride in the edema fluids studied above. Loeb and his co-workers (36) have demonstrated that water and salt are retained together in a normal individual after a period of salt deprivation in a manner qualitatively similar to that in which salt and water are retained in the nephrotic patient; the quantitative difference in response in the two patients is explained on the basis of the lower osmotic pressure of the plasma of the patient with nephrosis. From our data we are led to agree with Loeb and his co-workers (36) that " the facts . . . do not support the idea chiefly advanced by the school of Widal, Magnus-Levy and Blum, that the determining abnormalities in nephrotic edema are to be found in specific ion disturbances of renal or tissue behavior."

The importance of increased hydrostatic pressure within the capillaries as a cause of edema formation has been repeatedly stressed. The plethysmographic studies of Krogh, Landis and Turner (37) have shown that a relatively small increase above the normal in venous pressure is sufficient to cause measurable amounts of fluid to accumulate in the tissues. This conclusion was verified by Landis and his co-workers (38) who demonstrated that a venous pressure of 20 mm. Hg applied for thirty minutes was accompanied by a measurable loss of fluid from the blood stream. It would appear that the increased venous pressure which accompanies congestive heart failure is the important factor in the production of edema in these patients; the subnormal plasma protein values (Table I), and consequent low plasma oncotic pressure values (32) found in some cases are also of undoubted importance. In two of the above studied patients with congestive heart failure and edema (Table I) as well as in other similar patients who have been studied in this laboratory, the plasma protein values were reduced to the critical level at which patients with Bright's disease, but without congestive failure, may develop edema (8). It is not unexpected, therefore, that certain cardiac patients with only slight venous engorgement but with concomitant low plasma protein values form edema.

The venous congestion caused by carcinomatous obstruction, by cirrhosis of the liver, and by severe heart failure leads to the accumulation of fluids in the body cavities. Further, the protein concentrations found in certain of the chest and ascitic fluids (Table I) are sufficiently great to lower materially the difference in the osmotic pressure between these fluids and the blood plasma, with a consequent decrease in the relative reabsorption of fluids into the blood stream.

SUMMARY

1. A study of the distribution of electrolytes and certain non-electrolytes between blood serum and ascitic fluid, chest fluid. and subcutaneous edema fluid has been made in patients with nephritis, cardiovascular disease, carcinoma and cirrhosis of the liver.

2. The laws of chemical equilibrium which determine the relative concentrations of substances in the serum and chest, ascitic, and subcutaneous edema fluids appear to be the same, regardless of the site of fluid formation or the underlying pathological conditions.

3. The differences in the concentrations of the various electrolytes between the sera and fluids studied are governed by differences in the protein contents of these fluids. The concentrations of the non-electrolytes are practically equal in the sera and corresponding fluids when expressed in amounts per 100 grams of serum or fluid water.

4. The distribution of chloride and sodium between blood serum and the various edema fluids studied is in close agreement with the distribution expected according to Donnan's laws of membrane equilibrium. When the source of the blood sample is considered, the distribution of bicarbonate between serum and the various fluids likewise agrees with Donnan's law. The results do not indicate that bicarbonate is "bound" in the serum. Portions of the calcium, potassium, and inorganic phosphate apparently exist in the serum in the form of non-diffusible compounds.

5. Any changes in electrolyte concentrations which occur in the serum as a result of the administration of ammonium chloride, sodium bicarbonate, or salyrgan, are accompanied by similar compensatory changes in the electrolyte concentrations of the edema fluids.

6. This study of the distribution of electrolytes and non-electrolytes between serum and edema fluids substantiates the thesis that these edema fluids are simple dialysates in equilibrium with blood plasma.

7. In general, the protein concentration is lower in subcutaneous edema fluid than in chest or ascitic fluid. The total protein of serum is usually reduced in cardiac patients with edema.

8. In our patients with nephritis, cardiovascular disease. carcinomatous obstruction or cirrhosis of the liver, the protein concentrations of the different edema fluids bore no relation to the clinical diagnosis.

9. Theories of edema formation are discussed with reference to the results obtained in this investigation.

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THE MEASUREMENT OF THE ELASTICITY AND VISCOSITY OF SKELETAL MUSCLE IN NORMAL AND PATHOLOGICAL CASES; A STUDY OF SO-CALLED " MUSCLE TONUS "

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Muscle " tonus " has been described, defined, and measured in a multitude of different ways.[1] Scientifically speaking, however, there is no such *single* property of a muscle as its tonus. Rather tonus is a convenient term which includes many different properties such as elasticity, viscosity, and contractility to say nothing of the many nervous processes controlling the muscle reflexes. The continued employment of the term, convenient as it is, serves to avoid the necessity of analyzing " tonus " into its various components. An experimental analysis of this sort however is exactly what is needed for a complete understanding of the phenomenon and for an intelligent and scientific classification of the various types of rigidities familiar clinically.

Muscle " tonus " is so complicated in nature that it cannot properly be measured by any *one* method. Herein lies the justification for the development of yet another method. For scientific purposes, however, more is required than merely more methods, and more than merely a simple and convenient graphic test serving to distinguish different degrees or types of rigidity. For a scientific analysis each method must measure some one clearly defined physical property of the muscle in *absolute* units. Just as surely as a muscle has length and breadth it has elasticity and viscosity, etc. which may show functional changes, and these properties must be measured quantitatively, not qualitatively, in proper physical units. It is perhaps of interest to obtain drum tracings showing changes which may be interpreted as due to an increased muscle viscosity. It is better to measure this viscosity and discover how *much* it is increased.

METHOD

The method which we have developed and used has certain practical disadvantages in matters of convenience, but, like a previous method re-

[1] Recent methods of Pollock and Davis (1932) and of Berkwitz (1932) may be mentioned in this connection in addition to methods referred to in our previous paper (Smith, Martin, Garvey, and Fenn, 1930).

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ported from this laboratory (Smith, Martin, Garvey and Fenn, 1930) it does at least measure something definite in absolute units. The value of the apparatus is for research rather than for routine clinical use.

The method is a dynamic one and was developed in an effort to avoid the rather tedious calculations involved in our former method, which depended upon a mathematical analysis of the curve obtained by recording the rate of flexion of the knee in response to gravity. The general principle involved in this method is to move the leg passively to and fro, bending it at the knee at constant angular velocity, and to record on a revolving drum the force necessary to move it. The movement of the leg *at constant angular velocity* is an important feature of the apparatus because it permits us to neglect inertia. The force recorded is therefore purely one of resistance to displacement and is unaffected by acceleration or deceleration, and therefore unaffected by the mass of the leg.

The arrangement of the apparatus is shown in Figure 1. The subject lies on one side with one leg on a moving arm of the apparatus and the
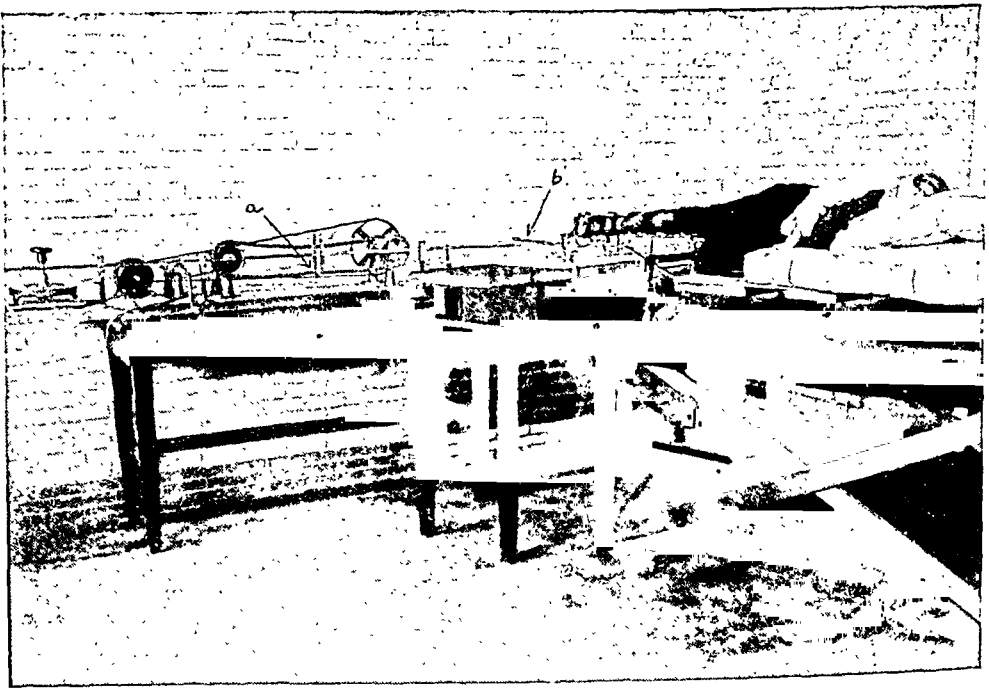
FIG. 1.   GENERAL VIEW OF APPARATUS

motor moves this arm back and forth carrying the leg with it. The axis of rotation of the moving arm coincides with the axis of rotation of the knee. Tension is recorded by a principle similar to the one used in the apparatus described by Schaltenbrand (1929). The details are shown in the diagram on Figure 2, there being two arms, A_1 and A_2 pivoted about the same vertical axis, this vertical axis being made to coincide with the axis of rotation of the knee. The leg rests on A_1 which is moved back and forth

by A_2 to which it is attached by springs (s). The displacement of A_1 with respect to A_2 caused by a given amount of force stretching the springs is a measure of the resistance to movement. This displacement is recorded by means of a thread attached to A_1, passing over a pulley P_1 which is fixed to A_2, and passing thence by another pulley P_2 to a lever under the table which records on a revolving drum. The string between P_2 and the lever coincides with the axis of rotation of A_1 and A_2 so that the movement
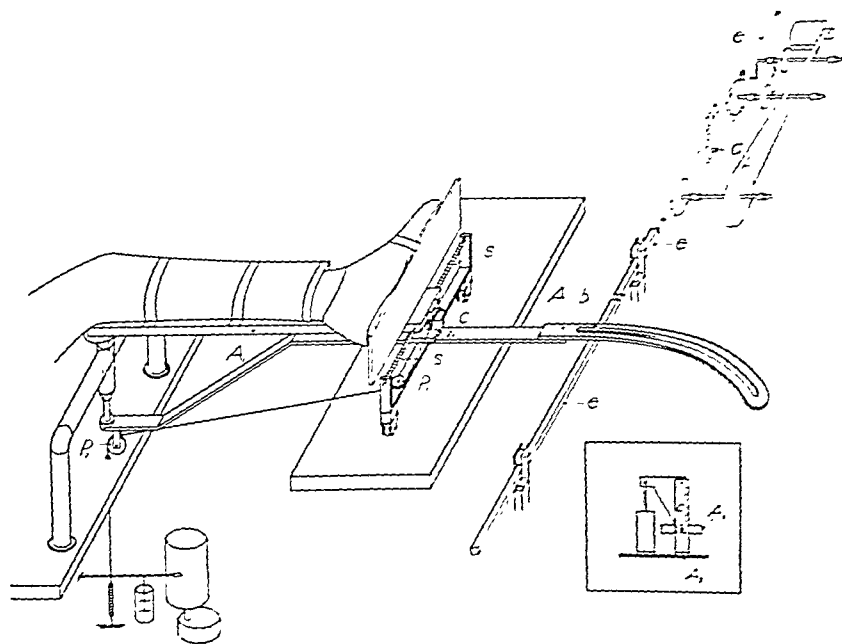
FIG. 2. DIAGRAM OF APPARATUS

Not drawn strictly to scale. The motor and chain in the upper right corner is much diminished in size. The inset shows details of the dash pot which is fastened to A_2 just to the right of the pin c where the thread over pulley P_1 is fastened. Rapid horizontal movements of A_1 and the leg in relation to A_2 are translated into vertical movements of the piston in the dash pot by means of a right-angled lever.

of these arms, without any real displacement of A_1 with respect to A_2, causes no movement of the lever. In order to support the weight of the leg, A_1 is provided with two wheels which run on a galvanized iron surface of the table with negligible friction (see Fig. 1). Likewise there is a wheel between A_1 and A_2 to reduce friction when the weight of the leg is bearing down on A_1. The force for moving the leg is applied to A_2 the lower arm, by a toggle b, which runs in a slot on A_2. b is carried backward and forward with constant linear velocity by the motor and the slot in A_2 is so designed that a linear movement of the toggle b results in movement of A_2

at constant angular velocity. The total excursion of b is 90 cm. and that of A_1 and A_2 and of the leg itself is 63°. The method by which the toggle b is made to move with a constant linear velocity is shown in Figure 1. The heavy chain passes over two sprockets which are driven by a ¼ horse power D.C. motor. The chain carries at one point a toggle a which engages with a slot in the bar c, which in turn carries the toggle b. (The bar c is shown in three sections in Figure 2. It is actually one steel bar 12 feet long and ½ inch square.) Thus at constant speed of rotation of the motor the toggle a on the chain moves with constant linear velocity and therefore also the toggle b. There is a considerable amount of vibration set up when this apparatus is running and this is damped out by two oil dash pots, one to damp movements between A_1 and A_2 and one directly on the recording lever. These effectively eliminate oscillations of high frequency but do not interfere with the slower changes due to the pull of the muscles.

Measurements of records

A typical record obtained with this apparatus is shown in Figure 3. The large spikes represent the points where the direction of motion is reversed. These spikes are of no significance, their height depending merely upon the speed of movement and the inertia of A_1 and the leg. Between

FIG. 3.   TYPICAL RECORD SHOWING CALIBRATION

Points where tonus is measured are indicated by arrows. Horizontal lines were drawn arbitrarily by spinning drum after record was taken. Reduced to ⅗ original size.

the spikes, the record indicates the total resistance to movement offered by the muscles, the joint, and any frictional forces in the apparatus. At the points marked by arrows measurements are made of the vertical distance above or below the horizontal base line. Deflections at these points represent the resisting forces at the end of the flexor and extensor movements respectively. The base line can be drawn representing zero tension but in practice we have found the position of this line slightly variable and have therefore drawn any base line arbitrarily merely for purposes of measurement and have utilized only the sum of the flexor and extensor resistances, i.e. the vertical distances on the record between two successive points indicated by arrows.

Routine measurement of the resistance to movement in the leg of a patient consists of a preliminary record at a very slow speed, then a series of

periods of four or five to and fro movements each, at increasing speeds, and finally a repetition of the same observations at decreasing speeds, ending up with a very slow movement. The speed is varied by the resistance in the motor circuit, but for very slow speeds it is necessary to turn the shaft by hand using a handle and crank provided for this purpose.

Figure 4 shows some complete sample records. C is a typical record from a normal subject. The large excursions, representing merely the over-shoot of the instrument when the direction is reversed, are naturally higher at high speeds. They appear likewise in the record A obtained with an artificial leg consisting merely of a weight tied to the apparatus. This record with an artificial leg differs however in showing practically zero deflections between and just previous to the large spikes, these being the points which are measured. Records D and E, Figure 4, are taken from subjects classified clinically as spastic (Parkinson's) and record B, from a case of muscular atrophy which was clinically flaccid.

After taking these records they are measured as described and the average values of deflections above and below the arbitrary base line obtained in successive movements at each velocity are determined and added together so that figures are obtained for the sum of the average flexor and extensor resistances at a series of different velocities. These deflections are calibrated in terms of kilograms on the original record (as in Figure 3) so that deflections in centimeters can be expressed in kilograms. These values are multiplied by the distances from the point on the apparatus where the calibrating force was applied to the axis of rotation, a distance of 60 cm. so that the results are finally expressed in terms of kilogram-centimeters of torque. These values are plotted against the angular velocity of movement in radians per second. Graphs representing average values of this sort are shown in Figure 5. As the velocity of movement increases the resisting force also increases, usually in a more or less linear fashion. It is noteworthy, however, that if these curves be extrapolated backwards they do not pass through the origin but intercept the X axis at a point which represents the force exerted at zero speeds of movement. It is, in fact, usual that a leg simply resting on the apparatus exerts some slight force either in the flexor or the extensor direction. This resting force may be regarded as a measure of the elasticity of the leg. The slope of the line, representing the increase of force for a given increase of velocity of movement, may be taken as a measure of the viscosity of the leg. The meaning of these forces may be stated more explicitly as follows:

Let the leg be assumed to be an elastic body which is at rest and exerts no external tension at some intermediate position. Let it have a coefficient of elasticity K and a coefficient of viscosity μ. If displaced a distance s_1 to the "flexor" end of the range the flexor muscles are stretched and an elastic force Ks_1 is exerted. Likewise an elastic force Ks_2 is exerted at the "extensor" end of the range. Since our measurements were all made at the ends of the range,
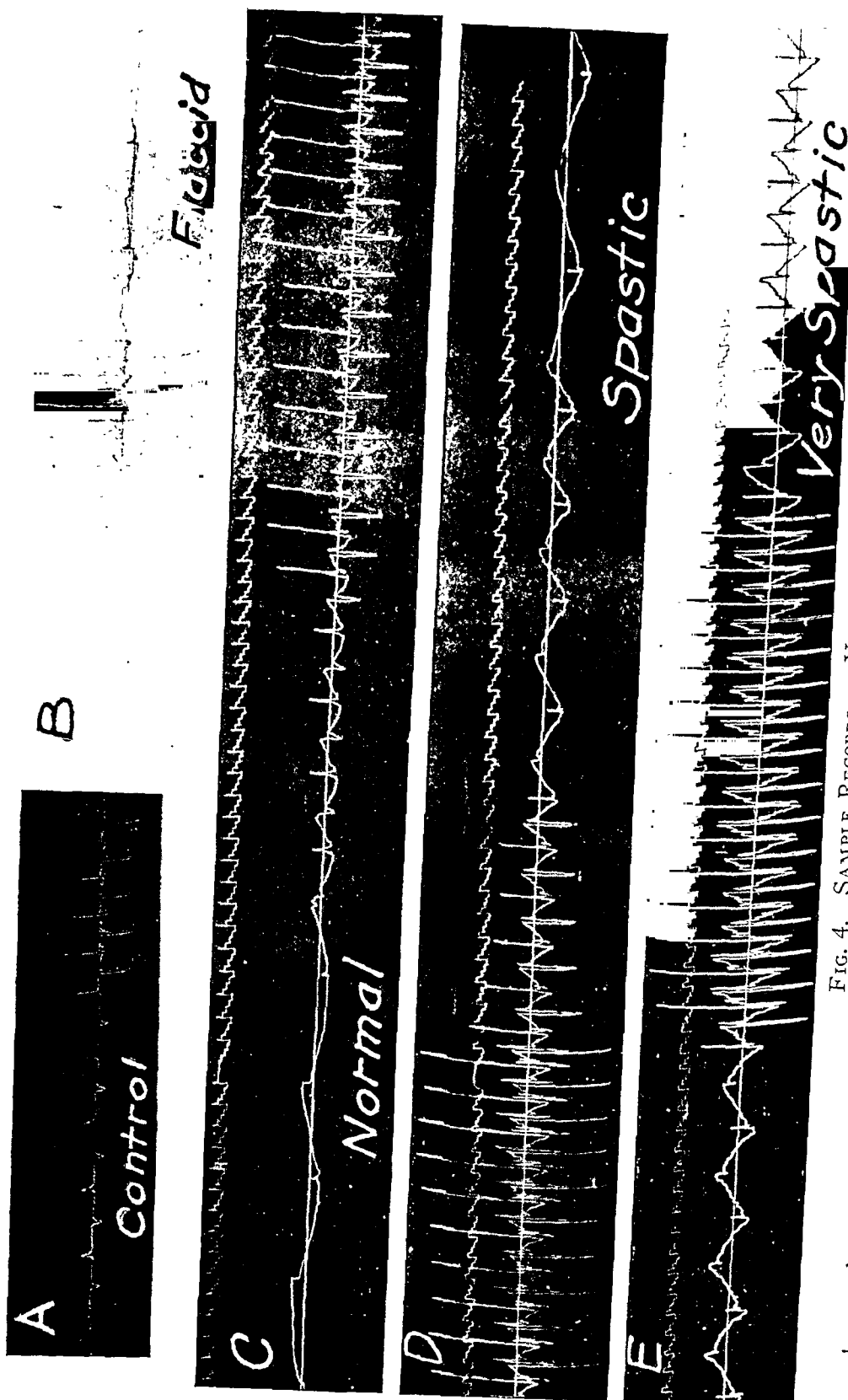
FIG. 4. SAMPLE RECORDS AT VARYING SPEEDS *A*, control—no leg; *B*, muscular atrophy; *C*, normal; *D* and *E*, Parkinson's syndrome. Time in 1 second intervals. Reduced to ¾ original size.

the resting force, or force at zero velocity (obtained by extrapolation of graphs in Fig. 4), is $Ks_1 + Ks_2$, or $2 Ks$, if $s_1 = s_2$. The force ($2 Ks$) is therefore proportional to the coefficient of elasticity of the leg. From this value the coefficient of elasticity of the muscles themselves could be obtained by dividing $2 Ks$ by 2 and by the average displacement. s. of the individual muscles and reducing to unit length and cross section area. For this purpose it would have to be assumed that the extensor muscles exert no force in the flexor position and vice versa. When the leg is moving with a velocity ds/dt there is an additional force measured equal to $\mu \, ds/dt$. The force which we have actually measured is 2
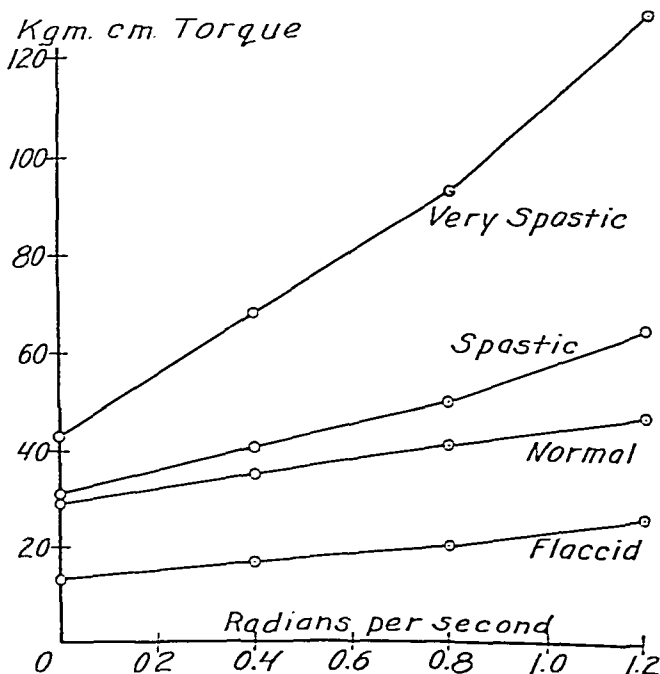
FIG. 5. AVERAGE VALUES FROM TABLE I

The resisting torque is plotted as ordinates against the angular velocity of movement of the leg at the knee in radians per second. The upper graph was taken from a patient showing Parkinson's syndrome.

$Ks + 2\mu \, ds/dt$. The slope of the graphs of Figure 4 is therefore equal to 2μ or twice the coefficient of viscosity. Lacking a knowledge of the true displacement. s, of the individual muscles we have not tried to calculate the coefficient of elasticity of the muscles themselves but we simply refer to the observed resting force as the elasticity of the leg. We shall show later, however, how an approximate calculation can be made of the value of μ for the muscles themselves. Treating the leg as a mere physical object this is a true coefficient of viscosity but it must be remembered that it includes "reflex viscosity" or the force developed as a result of proprioceptive reflexes initiated by the movement of the leg.

NORMAL AND PATHOLOGICAL CASES

We have measured in all the elasticity and viscosity of 19 normal legs, 46 flaccid legs, and 29 spastic legs (as classified clinically), and some others unclassified. Separate graphs for each of these legs, similar to those in Figure 5, have been plotted and figures obtained by graphical interpolation for the kilogram-centimeters of torque at 0.4, 0.8, and 1.2 radians per second. The difference between the values at 0.4 and 0.8 has been subtracted from the value at 0.4 in every case in order to obtain the force exerted at zero velocity (elasticity), this being in effect the backward extrapolation of the curve. The average values so obtained as recorded in Table I, and plotted in Figure 5, are 13, 29, and 31, respectively, for the flaccid, normal, and spastic legs. Likewise the average values for the slope of the curve between 0.4 and 0.8 and between 0.8 and 1.2 radians per second are also recorded in Table I. Here also the more spastic legs generally show the higher value, as would be expected.

TABLE I

Summary of torques measured in kilogram centimeters

	Number of cases	Torque at zero velocity	Increase in torque	
			Between 0.4–0.8 radians/second	Between 0.8–1.2 radians/second
Flaccid..........	46	13	3.6	5.7
Normal..........	19	29	6.0	5.5
Spastic..........	29	31	9.5	14.4

It is a point of some interest that this method does not invariably reveal abnormally high elasticity or viscosity in a leg which is classified clinically as spastic. Thus a group of 17 patients was studied. Nine of these exhibited symptoms of Parkinson's syndrome, and eight cases manifested spasticity due to a pyramidal tract lesion. Thus 34 legs were measured. An arbitrary scale was chosen so that they could be classified on the basis of the measurements as non-spastic, spastic, or markedly spastic. Likewise the same legs were classified on the basis of the clinical report in similar groups. The results may be summarized as follows:

Spastic clinically and by test 17
Spastic clinically, not spastic by test 14
Markedly spastic clinically, not spastic by test 6
Spastic by test, not spastic clinically 1

The result shows that 14 out of 31 clinically spastic cases do not appear to be so by this method of measurement. The largest percentage of failures to confirm the clinical impression of stiffness occurred in the group with

pyramidal tract signs where only one out of seven clinically spastic legs appeared abnormally resistant on the apparatus. Of 17 Parkinsonian legs which were rigid clinically, six failed to show any abnormality in the records as far as the viscosity was concerned but of these six cases, four showed abnormally high elasticity. Most of the spastic cases which the apparatus completely failed to detect were therefore due to pyramidal rather than extra-pyramidal lesions.

It may be suggested that the pyramidal spasticity is more directly or more completely dependent upon the stretch reflexes for its manifestation and that these stretch reflexes are not always elicited by our apparatus for the following two reasons: (1) There is a certain threshold speed of movement required for the development of a stretch reflex which is the basis of the pyramidal spasticity. This threshold is attained in the clinical test when the leg is moved by hand but is not attained in the laboratory examination. The threshold may be higher in pyramidal than in extra-pyramidal lesions. (2) The spasticity felt clinically may be in the form of an isolated stretch reflex which develops at a certain point in the excursion of the limb but is absent at the end of the excursion where measurements are made with our apparatus.

It is possible to offer another suggestion in partial explanation of these results. The psychological factor is an important one in measurements of this sort as Berkwitz (1932) has emphasized and the position of the subject in our apparatus is not always conducive to complete relaxation in all persons. This may account in part for wide variations in normal cases which thus overlap the spastic range and interfere with clean-cut distinctions. Further, considerable allowance must be made for subjective error in the rough clinical classifications.

The difference which we believe exists between pyramidal and extra-pyramidal spasticity is in agreement with the conclusion of Pollock and Davis (1929, 1932) who state that "muscles intoned by labyrinthine tonic reflexes show marked internal friction of so-called viscosity whereas the muscle intoned by muscle proprioceptors show an elastic type of curve." Schaltenbrand (1929) has also observed a difference between pyramidal and extra-pyramidal rigidity which would seem to indicate a greater dependence of the former upon stretch reflexes for he found that the rigidity persisted after the movement stopped in Parkinsonian muscles but not in muscles with spastic paralysis.

These results provide us with some information about the nature of the spasticity which is observed clinically. In some measure this is a static spasticity (elasticity) but in general the spasticity is only present when one attempts to measure it, that is, it is purely a dynamic spasticity (viscosity) which is developed to an abnormal degree by moving the leg. This spasticity becomes therefore to a considerable degree a matter of stretch reflexes. To progress further in the investigation of the subject it would be

desirable to be able to measure the threshold stimulus for these stretch reflexes or for a knee jerk.   Apparently in some spastic patients the threshold is sufficiently high so that no stimulus takes place at the velocities which we could attain in our apparatus.   These legs appeared to be completely normal as far as could be determined from graphic records, yet when tested in the usual clinical manner by simply bending the knee with the hand at considerably higher velocities, definite indications of spasticity could be obtained. The hand is more sensitive than the apparatus in this respect; at least it can test the muscle at higher velocities of movement and can pick out the particular range and conditions which will best elicit a spastic response.   In our previous study of this subject we found similar indications that clinical spasticity is a matter of stretch reflexes, for we found that the spastic leg did not fall smoothly but rather in jerks, so that the rate of fall could be well imitated by applying a tap to the patellar tendon while the leg was falling.   This appeared also in Parkinsonian legs which are therefore not uninfluenced by reflexes.

The inability of this method to detect spasticity in certain legs does not mean that it cannot detect small differences in tension but rather that the legs were not spastic under the conditions of the measurement—at least the spasticity was no greater than in the most resistant of normal legs.   There is in fact little doubt that a systematic investigation of two legs with our apparatus can reveal differences between them which cannot be detected by hand.   Thus one case of hemichorea was examined on five successive occasions and in four of these tests higher forces were measured on the affected side due either to higher elasticity or higher viscosity on that side. Another similar case was examined three times in two of which the affected side showed the greater resistance while the third trial showed no difference between the two legs.   None of these differences could be detected with certainty by the hand.

The records of Figure 6 were taken from a patient who readily went into a tetany on overbreathing.   Records *1, 2, 3,* and *4* were taken at intervals during a period of hyperventilation.   In *4* the tetanus was very marked and the resistance finally became so great that the motor was unable to move the leg at all.   The spikes indicating the sudden reversal of direction are scarcely visible at all on account of the extreme spasticity.

Normal viscosity

Figures in Table I for the slope of the curves representing torque plotted against velocity are of interest from the point of view of the coefficient of viscosity of normal muscle.   By coefficient of viscosity we understand the force necessary to maintain a unit increase in velocity of movement, i.e., dF/dV, where F represents the force in grams per cm.^{2} cross section and V is the velocity of shortening in cm. shortening per cm. length

of muscle per second.   It is possible to calculate from the figures of Table I a coefficient of viscosity expressed in these units for comparison with similar figures obtained in other ways.
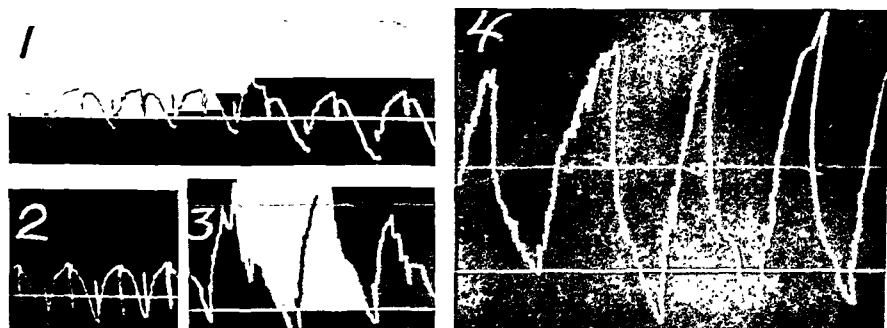
FIG. 6.   FOUR SUCCESSIVE RECORDS AT CONSTANT SPEED FROM A PATIENT DURING OVERBREATHING WHICH BEGAN JUST BEFORE THE FIRST RECORD WAS TAKEN.

The speed of the motor moving the leg was decreased somewhat in Number 4 because of the great resistance against which it had to work.   Time in 5 second intervals.   Reduced to ¾ original size.

To do this it is necessary to know the cross section area of all the muscles which are stretched when the knee is bent.   These comprise the quadriceps group, the hamstring group, and the gastrocnemius.   Only a rough estimate can be obtained of the average normal cross section of these muscles.   We have attempted to estimate it from cross section diagrams of the leg, such as those given by Braune and Fischer (1889, Table II).   The total cross section area of the diagram was measured by a planimeter and also the cross section area of the particular muscles concerned.   Knowing what fraction of the total cross section area is represented by a given muscle it is simple to calculate this fraction in absolute units from the circumference of an "average" normal leg.   In this way we have found that the cross section of the quadriceps represents 34.6 per cent of the total cross section of the leg, the hamstrings, 19.3 per cent, and the gastrocnemius 15.9 per cent.   The first two are measured at the upper third of the thigh and the gastrocnemius at the calf.   Thus the cross section areas may be estimated roughly at 790, 440, and 172 cm.^{2} respectively or a total of 1400 cm.^{2}.   From Table I the increase in torque between velocity 0.4 and 1.2 radians per second for normal muscles is 11.5 kgm. cm.   If the lever arm of the muscles concerned be estimated at 3.8 cm. (Fischer, 1927), then the force on the muscle tendons is 3020 grams or 2.15 grams per sq. cm.   This force is concerned with a velocity of rotation of the leg of 0.8 radians per second, which with a radius of 3.8 cm. means a rate of stretching of the muscles of 0.8 · 3.8 = 3.04 cm. per second.   If the muscle length be estimated at 40 cm., this means a velocity of shortening of 0.076 cm. per cm. muscle length per second.   The coefficient of viscosity is therefore 2.15 / 0.076 which equals 28.3.   This figure may be compared with considerably larger figures obtained by Bouckaert, Capellen and de Blende (1930), of 1120 by an extensometer method, 1015 by the Levin-Wyman method.   Similar values may be calculated from the data of Hill (1922) on work performed by human arm muscles in shortening at different speeds.

From Hill's data, Fenn, Brody, and Petrilli (1931, *see* their Table III) calculated the per cent loss of tension for an increase in the angular velocity of movement of 1 radian per second, and give also Hill's value for the maximum work, W_0 (in kgm. M.), which was performed. To calculate the coefficient of viscosity in the above units it is necessary to determine the force on the muscle corresponding to this maximum work, and to express the angular velocity in terms of cm. shortening per cm. of muscle. The work was performed by pulling a distance of 60 cm. so that $W_0/60$ is equal to the force on the hand at a distance of 34 cm. approximately from the elbow. If the lever arm of the muscles be taken as 2.88 cm. and the cross section of the flexor muscles be estimated as 29 per cent of the total cross section of the arm or about 218 sq. cm., then the force on the muscles is equal to $W_0/0.6 \times 34/2.88 \times 1000/218 = 90 \; W_0$ gm. per cm.² If the lever arm of the muscles is 2.88 cm. and the length of the muscle be estimated at 20 cm. then an angular velocity of 1 radian per second represents a shortening of 2.88/20 cm. per cm. length of muscle per second. If the values 43.5 K/W_0 (per cent loss of tension for an increase of 1 radian per second in angular velocity) as given by Fenn, Brody, and Petrilli be multiplied by 20/2.88, we have the per cent loss of tension for 1 cm. per second increase in velocity. Multiplying by 90 W_0 we have the coefficient of viscosity. Thus if W_0 equals 14 kgm. the coefficient of viscosity is $14 \times 90 \times 11.5 \times 20/2.88$ which is equal to 1010. Other values similarly calculated from the same data are 738 and 637. These values compare very well with those of Bouckaert, Capellen and de Blende, and also with others which we have obtained on stimulated frog muscle.

The value of 28 from our muscle tonus measurements is very much lower than similar coefficients of viscosity calculated from Hill's data (637 to 1010) and the difference is greater than the inaccuracies of the estimations. It is perhaps accounted for by the fact that in passively flexing a leg, the flexor muscles assist the movement by their own elasticity while the extensor muscles retard it and we are measuring really the difference between the flexor and extensor pulls. These relatively feeble elastic forces of resting muscles are of no importance in Hill's experiments with contracting arm muscles.

It is also of interest to compare the figures obtained in Table I for the resistance of normal muscles to stretching at different velocities with corresponding figures which may be derived from the data of Smith, Martin, Garvey and Fenn (1930) who measured muscle tensions from a record of the speed with which the lower leg falls from the horizontal position when suddenly released. In their Figure 6, subject number 2 (normal). graphs are given for the velocity of fall of the leg and for the resisting force at different times during the fall. From measurements on these curves, simultaneous values for velocity and force may be obtained and these are plotted in Figure 7 for times 0.1, 0.2, and 0.3 second after the moment of release of the leg. This gives a smooth graph through the origin showing that the force increases as the velocity increases. Three other points at low velocities are indicated by crosses on this curve. These were derived from the data of Table I. The torque at velocities 0, 0.4, 0.8, and 1.2 radians per second for normal muscles was divided by 3.8 cm., the estimated lever

arm of the muscle, to obtain the force in kilograms on the muscle itself. The value at zero velocity was substracted to get the force due to movement only. On the assumption that half of this force was measured during flexion and the other half during extension, the figures have been divided by two to give the tension in the extensor muscles at these three velocities. It is striking that the results obtained by these two utterly different methods should fall on the same smooth curve. The validity of the comparison depends upon the assumption that the dimensions of the legs
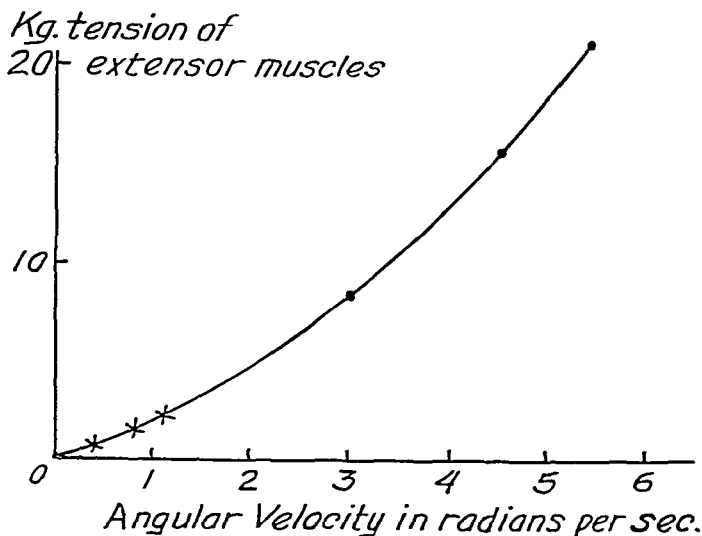
FIG. 7. RESISTANCE OF THE NORMAL LEG TO PASSIVE MOVEMENT (ORDINATES) PLOTTED AGAINST THE ANGULAR VELOCITY OF MOVEMENT (ABSCISSAE)

Dots are from the data of Smith, Martin, Garvey, and Fenn and crosses from data of Figure 5. The tensions have not been reduced to unit cross section areas.

in the two sets of data were approximately equal. One other complicating factor has been neglected, i.e. the length of the muscle, which has varied at the different points chosen. The graph nevertheless represents the best data now available on the resistance offered by human muscles to passive stretch at different velocities. Further data are necessary under more uniform conditions before conclusions can be drawn but the data as they stand indicate that the force increases approximately as the 1.26th power of the velocity of stretching, i.e. the curve is concave upwards. The same tendency is shown by the graphs of Figure 5.

DISCUSSION

We have described in this paper an apparatus with which we have been able to measure in absolute units the resistance to movement at the knee

joint at different velocities of rotation in both directions. Measurements have been made in both normal and pathological cases. The results show the variations of force as a function of velocity. Thus both a static and a dynamic measure of "tonus" is found, for the force at zero velocity is static and is proportional to the coefficient of elasticity of the leg. The increase of force with increase of velocity is a dynamic measure of "tonus" or is more properly defined as the coefficient of viscosity of the leg. This figure has been calculated and provides an adequate description of the behavior of the leg in the apparatus.

We do not believe that the apparatus described is ideal for the purpose. It has served, however, to measure the resistance to movement independently of inertia and as a function of velocity and the magnitude of this resistance is of physiological interest. On the clinical side the results serve to emphasize the importance of speed of movement in eliciting a spastic response. The reflex nature of spasticity is thus indicated. It appears that if we could explore the whole range of movement of the knee at different velocities we might expect to find some individuals in whom stiffness would only manifest itself above certain velocities and perhaps at certain knee angles.

SUMMARY

1. An apparatus is described which alternately flexes and extends the leg at the knee at constant angular velocities and records simultaneously the necessary torque which is applied to the leg for this purpose.

2. The force so recorded is not zero at zero velocity and this residuum is a static measure of muscle "tonus" and is found to be large in clinically spastic subjects. It is equivalent to a measure of the elasticity of the muscles.

3. The force (F) increases more or less linearly with increase in velocity of movement (V) and this rate of increase of F (dF/dV) is a kinetic measure of the muscle "tonus" and serves for a calculation of the coefficient of viscosity of the leg. The average value for normal legs is 28 grams per cm.² of muscle cross section for a velocity of stretch of 1 cm. per cm. muscle length per second.

4. Some legs classified clinically as spastic give normal values when tested by this apparatus. This occurs chiefly in patients with pyramidal rather than in those with extra-pyramidal lesions, and it appears to be due to the slow speeds of movement which are attained by the apparatus which are not adequate to reach the threshold for the stretch reflexes responsible for the spasticity observed clinically.

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THE IMMEDIATE RESPONSE OF THE PLASMA CHOLESTEROL TO THE INJECTION OF·INSULIN AND OF EPINEPHRINE IN HUMAN SUBJECTS[1]

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The present studies on the relation of insulin and of epinephrine to the cholesterol content of the plasma were carried out with the purpose of determining some of the factors which control the level of the plasma cholesterol in human subjects, especially in patients with diabetes mellitus. Previous reports from this laboratory have indicated the immediate effect (i.e., within three hours) of the ingestion of glucose (1), water and urea (2) on the cholesterol content of the plasma. The present experiments supplement the earlier investigations.

An increase in the blood urea after urea feeding usually resulted in a lowering of the blood cholesterol; it was concluded that a rise of the blood urea produces a decrease of the cholesterol concentration of the blood, either by altering the plasma colloid structure or that this reciprocal response was due to a compensatory osmotic process (2). However, the marked increase or decrease in the plasma cholesterol which occurs within a few hours following the ingestion of 100 grams of dextrose suggested the existence of additional influences, presumably metabolic in origin (1).

It seemed probable that the metabolic factors involved in altering the cholesterol content of the blood following the ingestion of glucose could be evaluated by a study of the accelerated oxidation of glucose in the body (insulin) and by observing the effect of an augmented mobilization of glucose from liver glycogen (epinephrine). It is true that insulin and epinephrine may also lower and raise the blood sugar, respectively, by processes other than those mentioned above, still it must be conceded that the increased oxidation of glucose by insulin and the mobilization of glucose from liver glycogen by epinephrine are the dominant influences of these hormones in altering the level of sugar in the blood.

LITERATURE

The effect of insulin on cholesterol and fat metabolism has been studied extensively. In brief, insulin lowered the blood cholesterol in normal dogs

(3, 4), in diabetic dogs (5), in rabbits (3, 6, 7), and in human subjects with "nephrosis" (8) and with diabetes mellitus (5, 9, 10, 11, 12); insulin had no effect on the blood cholesterol of normal dogs (5, 13); pancreatic extracts were ineffective in altering the blood cholesterol in rabbits (14); a rise in the blood cholesterol following insulin was not observed by any worker. Insulin inhibited the rise in the blood cholesterol in human subjects during ether anesthesia (15); its injection was followed by a decrease in the cholesterol content of the suprarenals in rabbits (14) and of the liver in diabetic dogs (16); it restored the fixation of cholesterol in the lungs and liver of the diabetic dog (17); it prevented the fatty infiltration of the liver in the dog following phloridzin poisoning (18). Insulin lowered the blood fat in diabetic subjects (19, 20) and diminished the fatty acid content of serum in "normal" and diabetic subjects (21).

The effects of the administration of epinephrine, of suprarenalectomy and of excitation and fear on cholesterol metabolism have been noted as follows: Epinephrine increased the blood cholesterol in human subjects (22, 23), in rabbits (3, 7, 24, 25, 26), and in dogs (3, 27); it lowered the blood cholesterol in human subjects (children) (28) and in dogs (29); irregular or no changes in the blood cholesterol following the administration of epinephrine have also been observed in rabbits (30, 31) and in dogs (13, 32, 33, 34). Unilateral suprarenalectomy in dogs (35) and rabbits (36), the extirpation of one and one-half suprarenals in dogs (32) and bilateral suprarenalectomy in rabbits (36) increased the blood cholesterol. On the other hand, unilateral or bilateral suprarenalectomy in rabbits (37) and extirpation of both suprarenals in rats (38) have been shown to be followed either by a decrease or by no changes in the cholesterol content of the blood. A case of unilateral suprarenalectomy in man is reported in the literature (39); the blood cholesterol showed no change. Recently, the effect of excitation and fear on the cholesterol content of the blood has also been noted; an increase in the blood cholesterol (40) and blood fat (41) was observed in cats exposed to barking dogs; sympathectomized cats showed no such effect (40).

MATERIAL AND METHODS

Diabetes mellitus. Ten subjects. Seven received 15 to 60 units of insulin intravenously; three 20 units subcutaneously.

Other clinical conditions. Thirteen subjects. Six received 6 to 20 units of insulin intravenously; one 20 units of insulin and 250 cc. of 10 per cent glucose intravenously; six 0.5 to 1.0 mgm. of epinephrine subcutaneously.

In every instance the experiment was carried out in the morning, no food having been taken since the preceding evening. The variations of the plasma cholesterol and the whole blood sugar were studied in many cases at intervals of 30, 45, 60, 90, 120 and 180 minutes after the administration of insulin and at intervals of 15, 30, 60 and 120 minutes following the injec-

tion of epinephrine.   Simultaneous hematocrit studies were made in twelve instances.   Six subjects received orange juice or glucose by mouth during the course of the experiment to offset hypoglycemic effects.

The cholesterol content of the plasma was determined by Sackett's method (42) using the modified procedure for colorimetric estimation employed in this laboratory (43); the whole blood sugar by the method of Folin and Wu (44).

Variations of the plasma cholesterol which deviated from the control by at least 7.8 per cent (twice the standard deviation observed in fasting subjects over a period of five hours (45)) were considered as noteworthy changes in the blood cholesterol following the administration of insulin or of epinephrine.

RESULTS

Insulin in diabetic patients.   Table I shows the variations of the plasma cholesterol and whole blood sugar following the intravenous or subcutaneous injections of 15 to 60 units of insulin in ten diabetic subjects.

A marked and rapid fall of the blood sugar may be accompanied by no significant alteration of the cholesterol content of the plasma (Cases 1, 2, 4, 7 and 8).   In Case 7, the intravenous injection of 60 units of insulin over a period of one and one-half hours resulted in a very marked fall of the blood sugar from 208 mgm. per cent to 54 mgm. per cent in three hours; the plasma cholesterol, however, remained constant.   Cases 6 and 9 showed so little variation in the cholesterol content of the blood as compared to control observations that they may be regarded as of no significance.   This leaves only three instances (Cases 3, 5 and 10) in which the changes in the plasma cholesterol were noteworthy: Case 3 showed an appreciable fall and a later rise of the plasma cholesterol, Case 5 a distinct increase, and Case 10 a marked fall.

Summary.   The administration of insulin to diabetic subjects was accompanied by no significant changes of the plasma cholesterol in seven of ten subjects.   A rapid diminution of the blood sugar from a distinctly hyperglycemic level to one of hypoglycemia effected by insulin may be associated with no change in the plasma cholesterol.

Insulin in non-diabetic patients.   Table II shows the variations of the plasma cholesterol and the whole blood sugar following the intravenous injection of 6 to 20 units of insulin in seven subjects with various clinical conditions other than diabetes mellitus.

No significant change in the plasma cholesterol followed the intravenous injection of insulin in five subjects (Cases 11, 13, 15, 16 and 17).   Cases 12 and 14 showed a definite rise and fall of the plasma cholesterol, respectively, following the injection of 10 units of insulin intravenously.   The cholesterol content of the plasma in Case 17 showed no change following the intravenous administration of 20 units of insulin and 250 cc. of 10 per

TABLE I

Simultaneous determinations of plasma cholesterol and whole blood sugar in ten subjects with diabetes mellitus following the intravenous or subcutaneous administration of insulin

Case number	Age	Sex	Units of insulin administered and route	Plasma cholesterol (mgm. per 100 cc.) / Whole blood sugar (mgm. per 100 cc.) Control	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes	180 minutes	Remarks	Percentage deviation of Highest cholesterol from control	Lowest cholesterol from control
	years												
1	46	F	15 intravenously	323 / *205*	326 / *90*		310 / *75*		312 / *105*			0.9	4.0
2	27	M	15 intravenously	338 / *326*	329 / *300*		326 / *242*		326 / *242*	329 / *217*			3.5
3	24	M	25 intravenously	294 / *183*	306 / *162*		263 / *108*		335 / *94*	352 / *85*		19.7	10.5
4	74	F	25 intravenously	497 / *150*	487 / *142*		536 / *83*	510 / *64*	490* / *172*		300 cc. orange juice after 90 minute sample	7.8	2.0
5	38	M	25 intravenously	211 / *213*	249 / *115*		277 / *58*		272 / *49*	263 / *58*		31.2	
6	24	M	35 intravenously	253 / *170*	255 / *148*		232 / *120*		238 / *71*	243 / *60*		0.8	8.3
7	50	M	60 intravenously	185 / *208*	180 / *188*	187 / *176*	182 / *143*	179 / *108*	180 / *77*	180 / *54*	Insulin given in divided doses 20 units every ½ hour for 3 doses	1.0	3.2
8	52	F	20 subcutaneously	192 / *268*	202 / *263*		190 / *250*	203 / *236*				5.7	1.0
9	50	F	20 subcutaneously	153 / *242*	147 / *238*		166 / *224*		146 / *183*			8.5	4.5
10	62	F	20 subcutaneously	235 / *213*	194 / *196*		227 / *210*		229 / *197*				17.4

* Excluded from the percentage deviation calculations; the ingestion of orange juice to relieve hypoglycemic symptoms preceded this determination.

TABLE II

Simultaneous determinations of plasma cholesterol and whole blood sugar in seven subjects with various clinical conditions other than diabetes mellitus following the intravenous administration of insulin

Case number	Age	Sex	Diagnosis	Units of insulin administered intravenously	Plasma cholesterol (mgm. per 100 cc.) Whole blood sugar (mgm. per 100 cc.)							Remarks	Percentage deviation of	
					Control	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes	180 minutes		Highest cholesterol from control	Lowest cholesterol from control
	years													
11	52	M	Arteriosclerosis	6	375 / 184	368 / 125	364 / 84					125 grams glucose by mouth 2 hours before insulin injection		2.9
12	18	M	Infectious arthritis	10	196 / 84	211 / 41		224 / 57		214 / 80	169* / 125	200 cc. orange juice after 120 minute sample	14.3	
13	52	M	Arteriosclerosis	10	460 / 114	479 / 25		441 / 58	387* / 94					4.1
14	35	M	Essential hypertension	10	215 / 71	210 / 66	186 / 54	205 / 43		208 / 86	186* / 180	200 grams glucose by mouth after 60 minute sample	3.7	
15	38	F	Sciatica	18	167 / 76	156 / 24	167 / 29					200 cc. orange juice after 120 minute sample		13.5
16	25	M	Peptic ulcer	20	105 / 79	186 / 31	191* / 40					200 cc. orange juice after 30 minute sample		6.5
17	64	M	Cerebral hemorrhage	20	252	257	255 / 263					Insulin given in 250 cc. 10 per cent glucose intravenously	4.3	4.6

* Excluded from the percentage deviation calculations; the ingestion of sugar in the form of glucose or orange juice to relieve hypoglycemic symptoms preceded these determinations.

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cent glucose. The onset of hypoglycemic symptoms (with extremely low blood sugar) was associated with no noteworthy alteration of the plasma cholesterol (Cases 12, 13, 14 and 16); the administration of sugar by mouth in the form of orange juice or of glucose to relieve these symptoms was accompanied by a distinct fall of the cholesterol in three out of four instances (Cases 12, 13 and 14).

Summary. The injection of insulin in subjects other than diabetics was not associated with significant alterations of the plasma cholesterol in five out of seven cases. The results were comparable with those observed in diabetic patients. Hypoglycemic reactions were not associated with fluctuations in the blood cholesterol; the administration of orange juice or of glucose to relieve these reactions resulted in a marked fall of the plasma cholesterol in several instances.

Epinephrine in non-diabetic patients. Table III shows the variations of the plasma cholesterol and the whole blood sugar following the subcutaneous administration of 0.5 to 1.0 mgm. of epinephrine in six subjects.

TABLE III

Simultaneous determinations of plasma cholesterol and whole blood sugar in six subjects with various clinical conditions following the subcutaneous administration of epinephrine

Case number	Age	Sex	Diagnosis	Amount of epinephrine administered subcutaneously	Plasma cholesterol (mgm. per 100 cc.) / Whole blood sugar (mgm. per 100 cc.)					Percentage deviation of	
					Control	15 minutes	30 minutes	60 minutes	120 minutes	Highest cholesterol from control	Lowest cholesterol from control
	years			*mgm.*							
18	28	F	Acute upper respiratory infection	0.5	170 / *48*	182 / *110*	160 / *102*	171 / *105*		7.0	5.8
19	32	F	Asthma	0.6	176 / *88*	217 / *105*	213 / *138*	196 / *99*		23.3	
20	31	M	Acute upper respiratory infection	0.6	335 / *102*	316 / *114*		319 / *118*	309 / *102*		7.7
21	35	F	Addison's disease (?)	0.6	263 / *85*	252 / *107*	255 / *136*	268 / *190*		1.9	4.1
22	34	M	Chronic alcoholism	0.8	231 / *73*	219 / *84*	219 / *102*	218 / *122*	217 / *99*		6.1
23	52	M	Arteriosclerosis	1.0	242 / *138*		242 / *144*	260 / *148*	261 / *164*	7.8	

In five subjects there was no appreciable change in the plasma cholesterol, although the blood sugar rose distinctly (Cases 18, 20, 21, 22 and 23). Case 19 was the only one of the six studied in which the plasma

cholesterol varied significantly; this case showed a marked rise of the cholesterol following the injection of 0.6 mgm. of epinephrine.

Summary. The subcutaneous administration of epinephrine in doses large enough to increase appreciably the sugar content of the blood was associated in five out of six instances with no change in the plasma cholesterol. One subject showed a significant rise.

Blood corpuscle volume per cent as influenced by insulin and by epinephrine. Table IV demonstrates the variations of the corpuscle volume of the blood in seven subjects following insulin administration and in five after the injection of epinephrine. In five cases, three receiving insulin

TABLE IV

The effect of insulin and of epinephrine on the corpuscle volume per cent (hematocrit) of the blood

Case number	Experiment	Corpuscle volume per cent (Hematocrit)								Remarks
		Control	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes	180 minutes	
3	25 units insulin intravenously	47		49		49		48	51	
5	25 units insulin intravenously	49		44		45		44	45	
6	35 units insulin intravenously	44		43		43		43	46	
11	6 units insulin intravenously	46		42	47					125 grams glucose by mouth 2 hours before insulin injection
12	10 units insulin intravenously	42		45		45		40	45	200 cc. orange juice after 120 minute sample
13	10 units insulin intravenously	47		47		44	43			200 grams glucose by mouth after 60 minute sample
14	10 units insulin intravenously	41		41	33	40		40	43	200 cc. orange juice after 120 minute sample
18	0.5 mgm. epinephrine subcutaneously	41	39	35		38				
19	0.6 mgm. epinephrine subcutaneously	55	48	54		51				
20	0.6 mgm. epinephrine subcutaneously	44	44			43	43			
22	0.8 mgm. epinephrine subcutaneously	42	45	42		44				
23	1.0 mgm. epinephrine subcutaneously	46		48		48	48			

and two epinephrine, a moderate to a marked fall of corpuscle volume per cent was noted (Cases 5, 11, 14, 18 and 19). Seven subjects showed little or no alteration of the percentile corpuscle volume of the blood. It is interesting to note that in two cases which demonstrated a definite increase of the blood cholesterol (one following insulin and one following epinephrine) an appreciable fall of corpuscle volume per cent was observed (Cases 5 and 19). For the most part, the fluctuations of the percentile corpuscle volume of the blood did not parallel the variations of the plasma cholesterol; Case 14 was exceptional. The marked fall of percentile corpuscle volume in the forty-five minute period was accompanied by a distinct fall of the blood cholesterol.

DISCUSSION

In direct contrast to the uniformity of insulin action on the blood sugar is the inconstancy of its effect on the plasma cholesterol; the cholesterol may rise or fall but usually remains unchanged for several hours following the parenteral administration of insulin.

An analysis of the literature regarding the effect of insulin on the blood cholesterol in diabetic subjects leaves no doubt that over a period of days and weeks insulin therapy is usually accompanied by a reduction of the hypercholesterolemia. However, it is only the immediate result of a single injection of insulin that is relevant here. There are only two publications that concern themselves with this phase of the problem. Nitzescu et al. (5) reported a decrease of the blood cholesterol in two diabetic subjects three hours after the injection of 20 units of insulin; the result, in either case, was not striking if one allows for the appreciable physiological fluctuations of the blood cholesterol (45). Recently, Sunderman, Austin and Williams (12) noted the changes in the concentration of the blood cholesterol following the injection of insulin in seventeen diabetic subjects. They gave 50 to 160 units usually at one injection, and studied the serum cholesterol when the blood sugar had fallen to normal or signs of insulin reaction were evident. They stated that with the fall in the sugar, the serum cholesterol consistently decreased; again, if one allows for the diurnal variations of the blood cholesterol (45), their protocols demonstrate an appreciable fall in only four instances.

It was hoped that some distinct contrast might be observed between the effect of insulin on the blood cholesterol in the diabetic and non-diabetic subject; none, however, was observed. The observations recorded in this paper would indicate that insulin, administered in the doses usually employed in the treatment of the chronic diabetic has no immediate effect upon the cholesterol content of the blood, even though there is a marked lowering of the blood sugar.

The reports in the literature concerning the effects of epinephrine on the blood cholesterol, as previously outlined, show little uniformity; our

own results demonstrate that in most instances epinephrine is without effect on the plasma cholesterol. Apparently, the increased glycogenolysis in liver and muscle brought about by epinephrine and resulting in hyperglycemia is accompanied by little or no change in the concentration of cholesterol in the plasma.

For many years, clinicians have believed that the signs and symptoms of insulin reaction referable to the vascular system, such as heart palpitation, tremor, pallor, sense of pressure over the sternum and the rise in blood pressure were due to a reflex hypersecretion of epinephrine. The recent work of Kugelmann (46) indicates that this regulatory output of epinephrine probably occurs during hypoglycemia. This worker injected ergotamine tartrate with insulin in ten patients and obtained a hypoglycemic condition without the circulatory signs described above in four instances. Attention is called to this phase of the problem, since the lack of any noteworthy changes in the plasma cholesterol during profound hypoglycemia, as observed in most of our cases following insulin, would indicate that the injection of epinephrine, per se, would be ineffective in altering the cholesterol content of the blood; such lack of effect, as we have shown above, was observed in five out of six instances.

CONCLUSIONS

The plasma cholesterol may occasionally rise or fall but usually remains unchanged following the administration of a single dose of insulin to diabetic and non-diabetic subjects. A rapid diminution of the blood sugar from a distinctly hyperglycemic level to one of hypoglycemia is, as a rule, associated with no significant change of the plasma cholesterol; the ingestion of orange juice or glucose during profound hypoglycemia usually results in a marked fall of the plasma cholesterol. The administration of epinephrine in doses large enough to increase appreciably the sugar content of the blood is usually accompanied by no significant change in the plasma cholesterol. Insulin and epinephrine, separately, often produce a transient diminution in corpuscle volume per cent, but the fluctuations in the percentile corpuscle volume of the blood bear no uniform relation to the plasma cholesterol content.

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STUDIES OF PHOSPHORUS OF BLOOD. II. THE PARTITION OF PHOSPHORUS IN BLOOD IN RELATION TO THE CORPUSCLE VOLUME

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The phosphorus content of whole blood is made up of phospholipids, which are insoluble in acid solution; inorganic phosphate and organic phosphate esters which are extractable with acid. The amount of nucleic acid phosphorus in normal blood is too small to be determined by present methods; the total phosphorus of blood as determined directly is within experimental error of the value obtained by the sum of the lipoid, inorganic and ester phosphorus values. The organic acid-soluble or ester phosphorus fraction is carried almost wholly in the corpuscles. Byrom and Kay (1) have stated that the amount of ester phosphorus per 100 cc. of erythrocytes is constant even when the volume of corpuscles varies widely from the normal, as in polycythemia or anemia. An exception to this rule has been noted by Kay (2), who found that reticulocytes and leukocytes contain much larger amounts of ester phosphorus than do normal erythrocytes, so that a marked increase in either of these types of cells in blood would be accompanied by an increased ester phosphorus. Kay also observed that reticulocytes and leukocytes contain appreciable amounts of nucleoprotein phosphorus, so that amounts of nucleic acid phosphorus, roughly proportional in quantity to the extent of the reticulocytosis or leukocytosis may be demonstrated. It is to be expected, then, that the presence in blood of reticulocytes or leukocytes in sufficient quantity to alter the level of ester phosphorus materially would probably also increase the quantity of nucleic acid phosphorus to a value readily demonstrable by present methods.

In the course of a study of the phosphorus of blood of normal children during growth (3), it was observed that changes in blood cell volume were not necessarily accompanied by equivalent changes in ester phosphorus. For example, the corpuscle volume drops from about 50 per cent to around 30 per cent within the first month of life, yet the ester phosphorus of the corpuscle increases during this period. The amount of increase is insufficient to compensate for the sharp drop in corpuscle volume, however, so that the amount of ester phosphorus per 100 cc. of whole blood is decreased. During early adolescence, changes of the reverse order occur. The corpuscle volume increases slowly, but the concentration of ester phosphorus in

the corpuscles decreases so rapidly that the whole blood values are decreased. No evidence of nucleic acid phosphorus was observed in any of these bloods.

This report is a study of the phosphorus partition in the blood of adults during rapid and marked changes in the percentages of corpuscles present. Two subjects were studied in detail; a patient with a severe anemia secondary to hemorrhage who showed a marked increase in red blood cells under iron therapy, and an individual with polycythemia vera, whose blood cell volume fluctuated widely under phenylhydrazine treatment. Two other patients with polycythemia were studied briefly. Serum calcium and plasma phosphatase were determined in addition to the phosphorus partitions, as these components of blood are closely related to phosphorus metabolism.

METHODS

Blood was collected after a 10 to 12 hour fast. The percentage of corpuscles was determined with the Van Allen hematocrit, using oxalated blood and centrifuging for 10 minutes at 3500 r.p.m. The phosphorus partition was determined according to the procedure outlined in a previous publication (3) using the Fiske-Subbarow technique for the development of color. (4). When the corpuscle volume of blood was much decreased, correspondingly larger blood samples were used for analysis. The phosphorus content of corpuscles was calculated from the values obtained for whole blood and serum, and from the percentages of corpuscles present. Plasma phosphatase was determined by the method of Jenner and Kay (5) and serum calcium according to the Kramer-Tisdall method (6). The Newcomer method was used for hemoglobin determinations (7).

RESULTS

The findings are given in Table I and in Charts 1 and 2. The normal values quoted for phosphorus partition are the averages from the blood of 7 healthy adults.

In all of the subjects studied, the most striking blood changes were in the ester phosphorus fraction. The lipoid phosphorus of corpuscles was increased in severe anemia, as has been previously noted by others (8). The serum phosphorus values were essentially normal throughout.

Ester phosphorus in anemia. The patient F. Z. suffered from a severe anemia secondary to repeated gastro-enteric hemorrhages. During the period of study very little loss of blood occurred, and the patient retained nitrogen, phosphorus and iron, and gained in weight. At the time of first observation, the anemia had been present for about 6 years. The corpuscle volume was only 9 per cent and the ester phosphorus of whole blood was 6.1 mgm. per 100 cc., less than one-third the normal value. The concentration of ester phosphorus in the corpuscles, however, was definitely above normal, although the increase was insufficient to compensate for the

TABLE I

Phosphorus partition in whole blood, corpuscles and serum, the serum calcium and plasma phosphatase in anemia and polycythemia. The phosphorus values are given in mgm. per 100 cc.

Name and diagnosis	Date	Corpuscle volume (per cent)	Whole blood — Total phosphorus	Whole blood — Lipoid phosphorus	Whole blood — Inorganic phosphorus	Whole blood — Ester phosphorus	Whole blood — Undetermined phosphorus*	Serum — Total phosphorus	Serum — Lipoid phosphorus	Serum — Inorganic phosphorus	Serum — Ester phosphorus	Corpuscles — Total phosphorus	Corpuscles — Lipoid phosphorus	Corpuscles — Ester phosphorus	Plasma phosphatase (units)	Serum calcium (mgm. per 100 cc.)	Hemoglobin (grams per 100 cc.)	Erythrocytes (millions)	Leukocytes	Reticulocytes (per cent)
Normal		43	37.0	11.1	3.1	22.3	0.5	13.1	9.0	3.6	0.5	68.5	14.0	51.2	6.5	10.8				
F. Z. Anemia (hemorrhage)	November 21, 1932	9	18.0	8.6	3.8	6.1	0.5	9.8	5.6	4.2	0.1	100.9	38.9	66.6	13.1		1.60	1.78	7,700	8.6
	December 2, 1932	15	26.6	8.7	2.6	15.1	0.2	12.3	8.1	3.9	0.4	108.0	12.7	98.7	6.1	9.5	2.32	1.80	4,950	3.3
	December 28, 1932	17	29.1	10.0	3.9	15.3	0.1	13.1	8.3	4.9	0.3	119.8	19.7	88.5	4.2	9.3	4.17	2.77	6,700	5.9
	January 13, 1933	25	34.5	9.6	4.5	20.5	0.1	15.3	9.0	5.1	1.0	92.1	11.4	79.0	5.1	9.2	6.21	3.70	7,950	3.2
L. H. Polycythemia osteomyelitis	November 25, 1932	56	48.0	9.8	3.3	35.2	0.3	14.0	9.8	3.8	0.4	74.6	9.8	62.8 (54.4)†			20.74	6.72	10,800	3.0
	January 13, 1932	47	41.9	12.6	3.8	25.5	0.2													
	December 7, 1932	49	34.2	11.1	3.3	19.6	0.2	12.7	8.5	4.0	0.3	68.0	15.1	49.7	6.9	11.2	15.25	5.38	11,550	1.8
	December 14, 1932	48	26.7	10.5	3.1	12.9	0.2	12.5	7.7	3.4	1.4	91.4	23.3	65.3	5.4	11.0	12.91	4.18	9,700	2.4
	December 28, 1932	31	37.9	12.1	4.7	20.9	0.2	12.4	8.8	3.4	0.4	94.8	19.3	66.5	6.0	10.6	6.28	2.34	19,400	6.5
	April 18, 1933	55	44.1	11.7	2.5	29.5	0.4	12.7	9.0	3.3	0.5	70.0	13.8	53.2	7.4	10.8	8.26	3.03	6,950	8.2
	July 21, 1933	55	47.1	11.8	3.2	31.8	0.3	11.2	7.2	3.2	0.7	76.5	15.7	57.2	8.5	11.0	14.55	6.54	9,850	0.6
	August 3, 1933	49	45.9	11.6	3.9	30.6	0.2	12.7	8.3	3.7	0.9	80.2	15.1	61.5			17.14	5.64	7,200	1.6
L. E. Polycythemia	June 24, 1933	65	59.3	15.0	4.0	40.5	0.2	11.7	6.2	5.0	0.7	84.9	19.7	61.9	8.1		16.92	9.05	15,200	
	June 28, 1933	63	65.3	17.1	4.3	43.7	0.3			5.5	1.1			68.7						
	July 1, 1933	63	65.8	15.0	3.4	47.1	0.3	14.1	7.6	4.7	1.8	96.2	19.3	76.3			19.30	9.24		
	October 16, 1933	54	48.0	12.4	2.8	32.7	0.1	11.2	7.3	3.5	0.6	79.3	16.7	60.4	6.8					
K. J. Polycythemia, syphilis, spinal cord tumor	July 21, 1933	62	63.6	15.5	3.0	43.5	1.6	12.1	7.5	4.0	0.2	112.4	20.1	70.2	10.2	10.8	19.54	7.5	40,000	5.4
	September 12, 1933	38	50.4	12.3	3.1	29.8	5.2			3.9	0.9			76.9		9.4				

* Difference between the total phosphorus by direct analyses and the sum of the determined components.
† Estimated.
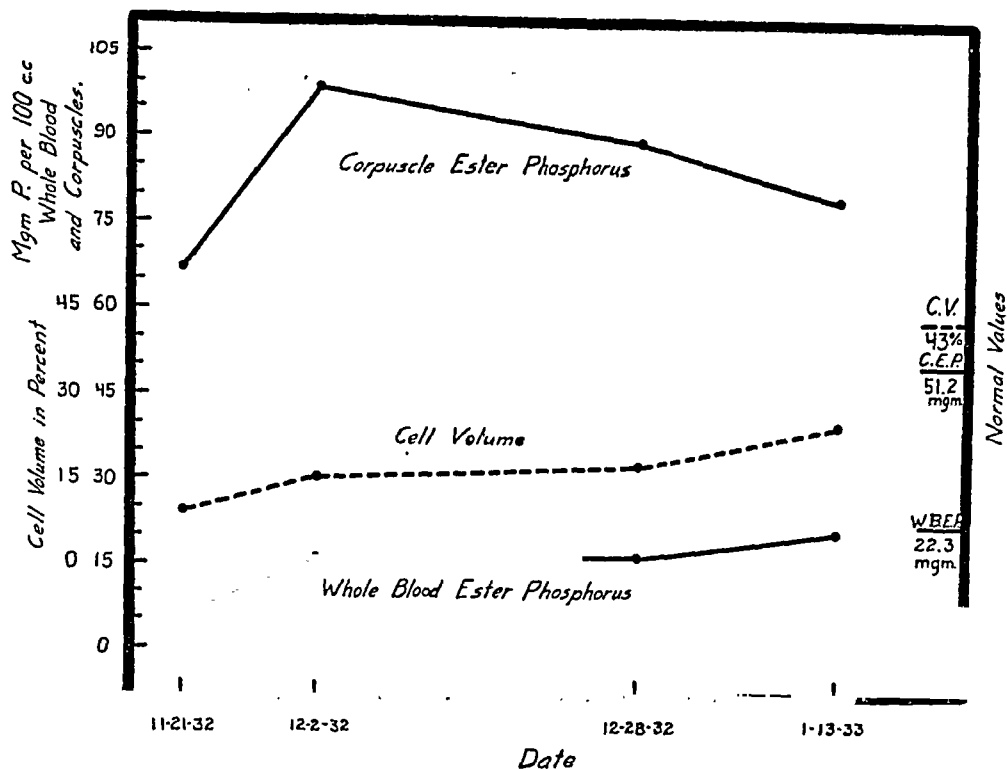
CHART 1.   THE CHANGES IN CELL VOLUME AND ESTER PHOSPHORUS OF WHOLE
      BLOOD AND CORPUSCLES OF THE ANEMIC PATIENT F. Z.
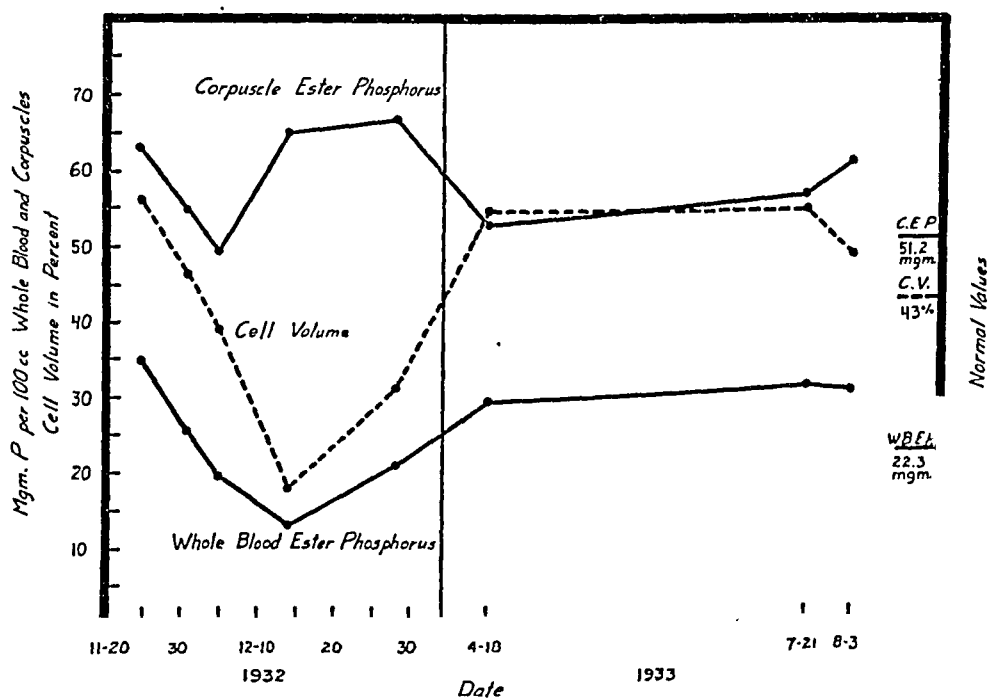
CHART 2.   THE CHANGES IN CELL VOLUME AND ESTER PHOSPHORUS OF WHOLE
      BLOOD AND CORPUSCLES OF THE POLYCYTHEMIC PATIENT L. H.

decreased quantity of corpuscles. With the increase in the quantity of erythrocytes and the concomitant improvement in the nutritional state, the retention of phosphorus was apparently sufficient to allow marked increase in the ester phosphorus content of corpuscles. Within 2 weeks, the value was nearly double the normal, though still insufficient for compensation. When the corpuscle volume had increased to 25 per cent, the ester phosphorus of whole blood became approximately normal. The amount carried per corpuscle, however, had decreased. This might seem to indicate that in anemia the ester phosphorus concentration in corpuscles may be increased only enough to make the whole blood value normal.

It will be noted that the undetermined phosphorus, the difference between the total phosphorus and the sum of the determined components, was never greater than the experimental error of the methods used. The amount of nucleic acid phosphorus present in blood was therefore negligible. The percentage of reticulocytes was never greater than 8.6, and the highest ester phosphorus values were observed when the percentage of reticulocytes was less than 5. The white cell count was normal. The increase in ester phosphorus content of corpuscles, therefore, cannot be ascribed to amounts carried by other types of cells than normal erythrocytes. It seems evident that a marked increase in the quantity of phosphorus carried by red cells is quite possible in the human adult.

Blood phosphorus in polycythemia. Three subjects were studied who showed an increase in corpuscle volume. Two of these suffered also from other diseases, but the results obtained in the 3 patients were so consistent that the abnormalities have been considered characteristic of polycythemia. Each of the three showed a marked increase in the phosphorus content of whole blood, especially in the ester phosphorus fraction, which was nearly double the normal value in 2 of the 3 subjects. The lipoid phosphorus of these 2 patients was also above normal, but that of the third subject (L. H.) remained within normal limits. The serum inorganic phosphorus of one subject (L. E.) was increased at the first 2 observations, but decreased when the corpuscle volume decreased. Serum inorganic phosphorus of the other 2 subjects remained normal. Other investigators (9, 10) have observed that serum inorganic phosphorus is not altered in polycythemia. The high value observed in subject L. E. may not have been related to polycythemia.

A study of the corpuscle phosphorus of the patients with polycythemia shows that the amount of ester phosphorus carried per 100 cc. of corpuscles was definitely above the normal in all 3 patients. This factor, together with the increased quantity of corpuscles, results in the extraordinary increase in ester phosphorus observed in whole blood. These patients, then, not only exhibited no decrease in amount of ester phosphorus carried in the corpuscles in order to compensate for the increased quantity of corpuscles, but on the contrary, the whole blood phosphorus was altered still

further from the normal by the excessive concentration of phosphorus in the corpuscles.

The patient L. H. was studied during a period wherein the corpuscle volume decreased from 56 to 18 per cent after treatment with phenylhydrazine, and also at intervals during the subsequent rise of the corpuscle volume. As the cell volume altered, the whole blood phosphorus tended to change in the same direction, the changes being due very largely to alterations in the ester fraction. Serum phosphorus values remained normal throughout, with the exception of one high serum ester phosphorus value which was noted simultaneously with a high white cell count. In the corpuscles, the lipoid phosphorus tended to vary inversely with the corpuscle volume so that the lipoid phosphorus of whole blood remained fairly constant throughout. The ester phosphorus of corpuscles, which was definitely above normal before phenylhydrazine treatment, apparently tended to approach the normal value as the corpuscle volume decreased, and remained close to the normal value until the severely anemic level was reached. When the cell volume dropped to 18 per cent, the corpuscle ester phosphorus had risen to 65.3 per cent, a value approximately that observed before treatment. The increase, however, was not nearly as large as that noted with the anemic patient F. Z. when the cell volumes were similar. The corpuscle ester phosphorus remained high during the anemic period. Three and one-half months later, the corpuscle volume was again above normal, but the ester phosphorus content of the corpuscles had dropped to within normal limits. Within the next 3 months the ester phosphorus values had again increased to the high levels found at the beginning of the study. An increase in corpuscle ester phosphorus thus accompanied each alteration from the normal corpuscle volume.

With this subject as with the anemic patient, no evidence of nucleic acid phosphorus was found. The percentage of reticulocytes was never high and a white cell count of 20,000 per c.mm. was not accompanied by any marked changes either in " nucleic acid phosphorus " (undetermined phosphorus) or in the ester phosphorus of corpuscles as compared with the value observed 2 weeks later when the white count had dropped to approximately 7000 per c.mm.

The corpuscle ester phosphorus values of the other 2 polycythemic patients were likewise always above the normal values. The subject K. J. is of interest because an appreciable amount of undetermined phosphorus, which may have been nucleic acid phosphorus, was found in her blood. The amount increased from 1.6 to 5.6 mgm. per 100 cc. of whole blood, during the time of study. The white cell count was markedly increased. This patient was so ill that it is difficult to ascribe the findings to any one cause, yet it seems worthy of comment that notwithstanding the high leukocyte count, the ester phosphorus values of the corpuscles were not

conspicuously higher than some of the values noted in the blood of patient L. E.

The above findings seem to indicate that an increased corpuscle concentration of ester phosphorus may be characteristic both of secondary anemia and of polycythemia. The increase may be compensatory in anemia, but the cause of the high content in polycythemia is not obvious. The number of patients studied is not sufficient to permit definite conclusions as to the quantities of ester phosphorus to be expected with a given cell volume, but it is felt that the results show very definitely that the concentration of ester phosphorus is by no means constant in the corpuscles of the human adult, and may show very marked fluctuations in the same individual under differing conditions of corpuscle volume.

Because phosphatase activity is associated with ester phosphorus, it seemed desirable to determine the plasma phosphatase. The values observed were wholly within the normal limits of 4 to 10 units per 100 cc. The serum calcium values of the patients with polycythemia were normal at all times. This is in agreement with the report of Benedict and Turner (12) but not in accord with the findings of Brown and Roth (10) who noted elevated serum calcium values in polycythemia, nor with the observation of Rabinowitch (11) who found lowered values in this disease. The serum calcium of the patient with anemia was near the lower limit of normal range, perhaps as a result of malnutrition accompanying the anemia.

The authors are indebted to Dr. C. W. Baldridge and to Dr. Adelaide Barer of the Department of Medicine, through whose cooperation these patients were studied.

SUMMARY

1. The phosphorus partition in blood, serum and corpuscles, the serum calcium and plasma phosphatase were determined in 4 patients with altered erythrocyte volumes. Two of these subjects were studied during a period of changing corpuscle volume.

2. The components most affected by alterations in corpuscle volume were the lipoid and ester phosphorus of the corpuscles. The former was increased in severe anemia, the latter fraction tended to be increased whenever the blood cell volume was markedly altered from the normal, regardless of the direction of the alteration.

3. It is concluded that the ester phosphorus concentration in human corpuscles is not constant.

4. No alterations in plasma phosphatase were observed coincident with the alterations in ester phosphorus. Calcium values were normal in the 3 patients with polycythemia, but slightly subnormal in an anemic subject.

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ANTIBODY RESPONSES IN INFECTIOUS MONONUCLEOSIS

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Until recently the importance of heterophile antibodies in clinical medicine was scarcely appreciated. A short time ago, however, these substances became of practical interest when Paul and Bunnell (1) observed the occurrence of heterophile agglutinins and hemolysins in four cases of infectious mononucleosis. Heretofore they had only been found consistently in patients previously treated with horse serum. Confirmation of Paul and Bunnell's report has lately been offered by Rosenthal and Wenkebach (2), Boveri (3), and Bunnell (4).

The purpose of this paper is to report the results of observations on the presence of heterophile antibodies in the sera of patients having infectious mononucleosis and other diseases, with special reference to the value of the test for diagnostic purposes, and also to determine whether or not other unrelated bacterial serological reactions are associated with agglutinins of sheep red blood cells.

MATERIALS AND METHODS

The methods are those described by Davidsohn (5) and, for the sake of uniformity, the results are tabulated according to his arrangement. Previous authors (1) have noted a close parallelism between the titers of agglutinins and hemolysins. In order to simplify the technique, only the agglutinins have been determined in this study. The following materials are required: the patient's serum, a suspension of sheep red cells, and physiological saline solution. The serum, obtained as for any agglutination, is inactivated for 15 minutes at 56° C.; kept in the icebox, its potency, so far as the agglutinins are concerned, remains constant over a period of several months. Dilutions of the serum starting with 1 to 4, are carried out as far as is indicated. Weekly collections of the sheep cells are made. These are washed three times; from them a 2 per cent suspension of packed cells is prepared. To each tube containing 0.5 cc. of diluted serum, 0.5 cc. of 2 per cent sheep cells is added. Finally, the addition of 1 cc. of saline brings the total volume to 2 cc.

The tubes are shaken and placed in a water bath at 37° C. for one hour; then kept overnight in the icebox. The following morning the

tubes are gently inverted three times after which the results are recorded as follows:

$+++$ a single mass of cells
$++$ large flakes
$+$ small flakes
$\pm$ barely macroscopic agglutination.

HETEROPHILE ANTIBODIES IN SERA FROM CASES OTHER THAN INFECTIOUS MONONUCLEOSIS

In the course of these studies heterophile antibody determinations were carried out in 300 adult patients on the medical service. Many of these tests serve as controls. However, three groups of diseases, which were deemed of particular interest in relation to the problem, were specially included. They were:

(*a*) Conditions with any clinical features similar to those of infectious mononucleosis.

(*b*) Conditions which are associated with bacterial agglutination reactions.

(*c*) Certain blood dyscrasias.

(*a*) Conditions with any clinical features similar to those of infectious mononucleosis

Some of the more pertinent examples of these are: diphtheria, secondary syphilis, streptococcus tonsillitis, Vincent's stomatitis, herpetic stomatitis, scarlet fever, mumps, chicken pox, measles, luetic cervical adenitis, miliary tuberculosis, erythema nodosum and multiforme, acute rheumatic fever, subacute bacterial endocarditis, pneumonia, erysipelas, influenza, poliomyelitis, tertian and quartan malaria, yaws, trichiniasis, pregnancy (all stages), obstructive jaundice, hyperthyroidism with lymphocytosis, symptomatic purpura, asthma, angioneurotic edema, and serum disease.

The distribution of titers in the miscellany of diseases studied is in Table I.

TABLE I

Distribution of heterophile antibody titers in 300 hospital patients

Titer	Less than 1 : 4	1 : 4	1 : 8	1 : 16	1 : 32
Per cent..........	29	32	25	14	0

These results conform in general with the data cited by Paul and Bunnell (1). No effort was made to correlate the values with the age group of the patients as these authors have done. The only titers higher than 1 to 16 were encountered in patients who, on further investigation, were found to have had horse serum within a period of less than a year.

The subject of serum therapy merits a word of mention. Davidsohn (6) has demonstrated the eventual appearance of heterophile antibodies in

human serum after administration of horse serum, which contains the heterophile antigen. These results were confirmed. Whatever may be the original titer of the patient's serum, this value will suddenly increase by three or four dilutions, in a period ranging from six to nine days, after the introduction of the serum, but declines once more to the former level over an interval of two to three months. The highest point attained in a series of 18 serum-treated individuals whom we followed, was 1 to 512, this being in a colored woman treated intramuscularly with anti-erysipelas serum. The preliminary titer of her serum had been 1 to 16. For our present purposes this emphasizes the importance of eliminating horse serum as the inciting agent before drawing any conclusions from an increased titer of heterophile antibodies.

(b) *Conditions which are associated with bacterial agglutination reactions*

For a reason that will become apparent later (*cf.* Case 16), a study was carried out with many of the diseases in which bacterial agglutination reactions are employed.

TABLE II

Heterophile antibody titers in a selected group of infections

Disease	Organism agglutinated	Titer	Sheep cell agglutinins			
			1:4	1:8	1:16	1:32
Typhoid fever.........	B. typhosus	1 : 1280+	+	—	—	—
Typhoid fever.........	B. typhosus	1 : 1280	++	++	—	—
Typhoid fever.........	B. typhosus	1 : 640	++	+	±	—
	B. paratyphosus A	1 : 160				
Typhoid fever.........	B. typhosus	1 : 1280	—	—	—	—
Typhoid fever.........	B. typhosus	1 : 1280	+	±	—	—
Paratyphoid (carrier)...	B. paratyphosus B	1 : 160	+	+	+	—
Malta fever...........	B. melitensis-bovine	1 : 2560	±	—	—	—
	porcine	1 : 320+				
	caprine	1 : 320+				
Dysentery............	B. dysentery Shiga	1 : 160	+	—	—	—
Dysentery............	B. dysentery Flexner	1 : 160	+	—	—	—
B. proteus pyelitis......	B. proteus X-2 and X-19	1 : 160	—	—	—	—
Tick-bite fever.........	B. proteus X-2 and X-19	1 : 160	±	—	—	—
Tularemia (typhoidal form)...............	B. tularense	1 : 320	—	—	—	—
Suipestifer sepsis.......	B. suipestifer, Group II	1 : 80	++	+	—	—

In not a single one of these cases did sheep cell agglutinins appear in increased titer. It will be recalled that the dysentery Shiga organism contains the heterophile antigen.

(c) *Certain blood dyscrasias*

One or more of each of the blood dyscrasias detailed in Table III were studied.

TABLE III

Heterophile antibody titers in some diseases of the blood

Disease	Sheep cell agglutinins				Remarks
	1:4	1:8	1:16	1:32	
Pernicious anemia	+++	±	–	–	One week after treatment begun. R.B.C. 1,400,000
Erythremia	+++	–	–	–	No therapy. R.B.C. 7,500,000
Paroxysmal hemoglobinuria	+++	±	–	–	
Chronic aplastic anemia (benzol)	+++	++	++	–	R.B.C. 2,500,000; Hb 45 per cent; platelets 80,000; W.B.C. 3,000
Sickle cell anemia	+++	++	++	–	R.B.C. 2,000,000; Hb 40 per cent; W.B.C. 12,000
Hemophilia	–	++	–	–	Clotting time 3 hours
Purpura hemorrhagica	+++	++	±	–	Platelets 100,000. *Cf.* Case 17, however
Hodgkin's disease	++	+	±	–	
Leukopenic infectious monocytosis (7)	++	+	–	–	W.B.C. 2,300; lymphocytes 37 per cent; monocytes 63 per cent. Recovered after pentnucleotide
Agranulocytic angina	–	–	–	–	W.B.C. 500, all plasma cells
Acute myeloblastic leukemia	++	±	–	–	W.B.C. 25,000; 40 per cent myeloblasts
Chronic myeloid leukemia	–	–	–	–	W.B.C. 37,000 (after irradiation)
Chronic monocytic leukemia	–	–	–	–	W.B.C. 10,800; 46 per cent monocytes
Lymphosarcoma	–	–	–	–	W.B.C. 7,000; 57 per cent lymphocytes
Acute lymphatic leukemia	–	–	–	–	W.B.C. 12,000; 95 per cent lymphocytes
Chronic lymphatic leukemia	–	–	–	–	W.B.C. 150,000; 95 per cent lymphocytes

The titers tabulated are representative values from individual cases. In none of these were the agglutinins found above normal levels.

HETEROPHILE ANTIBODIES IN SERA FROM CASES OF INFECTIOUS MONONUCLEOSIS

Fifteen cases fulfilling the clinical requisites of infectious mononucleosis, have been studied in some detail.[1]

In each case recorded in Table IV the agglutinin titer is the highest value obtained in the course of the disease, during the period of observa-

TABLE IV

Concentration of sheep cell agglutinins in the acute stage of fifteen cases of infectious mononucleosis

Case number	Name	Age	Day of disease	Temperature	W.B.C.	Differential			Heterophile antibody titer
						Pmn.	Lym.	Mon.	
		years	*days*	*° F.*	*per c.mm.*	*per cent*	*per cent*	*per cent*	
1	J. M.	19	5	103.0	10,500	30	70		1 : 256
2	R. H.	23	22(?)	101.0	10,700	40	60		1 : 1024
3	B. W.	23	6	102.4	5,680	46	51	3	1 : 1024
4	W. D.	16	12	100.8	17,500	29	71		1 : 2048
5	B. C.	6	13	100.0	12,000	45	47	8	1 : 4
6	L. W.	24	14	101.0	12,000	23	76	1	1 : 2048
7	E. M.	21	23	102.6	12,300	40	53	7	1 : 128
8	J. A.	14	26	99.0	8,050	30	66	4	1 : 32
9	M. G.	29	10	100.0	7,600	37	60	3	1 : 1024
10	B. K.	10	12	102.0	46,000	11	89		1 : 2048
11	M. B.	19	14	99.0	9,700	23	66	11	1 : 32
12	E. W.	6	13	101.0	12,000	52	40	8	1 : 16
13	R. B.	20	19	102.0	8,750	26	61	13	1 : 2048
14	F. C.	23	11	101.0	7,550	41	59		1 : 4096
15	H. C.	17	12	100.0	27,000	15	83	2	1 : 512

tion. In thirteen of the fifteen examples, heterophile antibodies were found present in the patient's serum to a titer of 1 to 32 or higher.

As soon as there exists any clinical basis for suspicion of the disease, sheep cell agglutinins will be found in abnormal concentrations in those instances where any increase is ultimately observed. This concentration rapidly attains a maximum after which there is a gradual decline, lasting between 6 weeks and 9 months, to normal levels even though this may be temporarily coincident with an aggravation of the symptoms of the disease. From observations on patients who have received horse serum, and, hence,

in whom the exact time of introduction of the heterophile antigen is known, the latent period, before the antibodies appear, is found to be six days or more. By analogy, then, the incubation period of infectious mononucleosis would be expected to be at least six days, which is in accord with clinical estimates.

The highest titer encountered in the group of fifteen cases was 1 to 4,096. This value seemed to bear no closer parallelism with the severity of the disease than do the titers, after serum therapy, conform with the degree or, indeed, even the presence of serum sickness. The greatest concentration of antibodies in a group of 18 serum-treated individuals occurred in a case in which the only evidence of serum sickness was a rise of temperature to 101.6° F. on the sixth day after institution of serum therapy. The fever was unaccompanied by any of the usual symptoms of serum sickness.

Brief summaries will be given of five of the cases of infectious mononucleosis, each one illustrating a point of interest.

Case 1. Antibodies preceding abnormal cells in the blood.

J. M., a white, male, medical student, aged 19, was admitted to the Johns Hopkins Hospital on October 4, 1932, complaining of a sore throat.

Six days before admission, enlarged cervical glands were noted. Two days later, sore throat and anorexia developed. Two days before entry, the patient had chills and fever.

On admission, the temperature was 100.6° F. The pharynx appeared dusky red; there was one hemorrhage on the soft palate. Marked general grandular enlargement was present and the spleen was palpable. The white blood cell count was 7,400 of which 47 per cent were polymorphonuclears, 45 per cent normal large lymphocytes and 8 per cent small lymphocytes. It was not until two days later that the characteristic abnormal large cells with irregular foamy nuclei made their appearance in the blood stream. Fever persisted for almost a week, temperature attaining a normal level on October 10. The leukocyte count rose to 14,240, falling within normal limits after October 14.

On the day after entry the agglutinin titer was 1 to 256. Subsequent determinations are presented in Table V, from which it may be seen that the heterophile antibodies were present in high titer for ten days. Subsequent tests, at intervals, showed a progressive diminution in the reactivity which reached a normal value 3½ months after the onset of the disease.

This case illustrates the typical behavior of the antibodies and also indicates that these may precede the appearance of abnormal white cells in the peripheral blood.

Case 2. Antibodies present in abnormal titer during long prodromal period.

R. H., a white, male, medical student, aged 23, was admitted to the Johns Hopkins Hospital on November 1, 1932, complaining of general malaise.

Onset of present illness occurred one week before admission, with a cold and malaise followed by an unproductive cough. Two days before entry, fever, prostration and anorexia put in their appearance.

On admission the temperature was 101.4° F. The pharynx was diffusely injected; the axillary, epitrochlear, cervical, and inguinal glands were slightly en-

TABLE V

Relationship between heterophile antibody titer and the clinical course of a mild case of infectious mononucleosis (Case 1)

Date	Sheep cell agglutinins								W.B.C.	Differential				Tempera-ture
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512		Pmn.	Ll.	Sl.	Mono.	
									per c. mm.	per cent	per cent	per cent	per cent	°F.
October 5, 1932	+++	+++	+++	++	++	++	++	—	10,500	30	66	4		103.0
October 7, 1932	+++	+++	++++	++++	+++	++	++	—	14,240	37	59	4		101.0
October 11, 1932	+++	++++	++++	++++	+++	++	+	—	8,160	33	60	7		99.0
October 14, 1932	++	+	+	++	+	±	—	—	9,920	35	55	10		99.0
November 16, 1932	+++	++	+	—	—	—	—	—	7,280	62	7	24	7	normal
January 13, 1933	++	++	+	—	—	—	—	—						normal
March 25, 1933	++	++	±	—	—	—	—	—						normal

larged and the spleen was palpable. The leukocyte count was 6,900 of which 81 per cent were polymorphonuclear neutrophilic leukocytes, 6 per cent eosinophiles, and 13 per cent lymphocytes. Four days later, the patient's temperature became normal. At this time the blood count was 10,200, polymorphonuclear neutrophils 79 per cent, eosinophils 1 per cent, lymphocytes 20 per cent. The patient was discharged on November 9, after being afebrile for five days, although a sore throat, which had appeared while he was in the hospital, persisted.

The diagnosis of this patient's condition was, at first, in doubt. The usual laboratory tests for enteric fever were negative. However, with serum obtained on November 2, agglutinins for sheep red blood cells were found to be present in a titer of 1 to 32. Even though this degree of serological activity is higher than the average normal, it was not deemed sufficiently significant to justify a diagnosis of infectious mononucleosis.

Three days after discharge his throat became more sore, fever returned and he was readmitted on November 14, three weeks after the onset of the initial symptoms. Temperature was 101° F., and glandular and splenic enlargement, noted on the previous admission, were still present. In addition there was a characteristic follicular tonsillitis as well as a mottled scarlatinal-like erythema over the body. However, the true diagnosis was now revealed by the hematological examination which showed a leukocyte count of 10,700, 48 per cent large and 12 per cent small lymphocytes, many of the cells typical of infectious mononucleosis being present. His course was a mild one. After four days of rehospitalization, the fever abated and remained normal. On November 25 with a blood count of 9,300 there were still 60 per cent lymphocytes, some of these abnormal in type.

On the day of the second admission serum was obtained in which the heterophile agglutinin titer was found to be 1 to 1,024. The hematological diagnosis was therefore confirmed by the serological test. Additional observations with samples of serum, obtained at intervals during convalescence, demonstrated a progressive decline in antibodies. The last test, made ten months after recovery, reacted only in a dilution of 1 to 8.

Interest in this case centers around the fact that the final outcome emphasized the significance of the early, slightly increased, heterophile antibody titer contained in the patient's blood. The serological titration made with the patient's serum at the time of the first undiagnosed stay in the hospital indicated, in all probability, the true nature of the illness. Additional experience with sera from other cases of infectious mononucleosis suggests that a titer of sheep cell agglutinins no higher than 1 to 32 is adequate, in most instances, to establish a diagnosis.

Case 3. Diagnostic significance of antibodies in a case without conspicuous adenopathy.

B. W., a white, female, student nurse, aged 23, was admitted to the Johns Hopkins Hospital on May 1, 1933, complaining of headache of five days' duration.

The illness began one day after the first inoculation with typhoid vaccine. It was characterized by fever, sweats, headache, dizziness and weakness. Her throat was not sore.

On admission, the temperature registered 102.4° F., the pulse, 84. She appeared prostrated. Small post-cervical, axillary and inguinal glands were palpable. The tip of the spleen could be felt. The leukocyte count was 5,680, polymorphonuclears 46 per cent, small lymphocytes 32 per cent, large lymphocytes 19 per cent, monocytes 3 per cent.

With blood obtained on the day of admission, the heterophile antibody titer was 1 to 1,024. Within five days the leukocyte count rose to 14,640 of which polymorphonuclears composed only 17 per cent. Normal temperature was attained on the eleventh day of the disease. On discharge one week later, the leukocyte count was 8,000 of which 60 per cent were lymphocytes and monocytes. During the stay in hospital, several cervical lymph glands became slightly more enlarged, but at no time were sufficiently conspicuous to indicate an acute adenopathy.

The results obtained with the blood of this patient are a striking example of the value of the heterophile antibody determination. The serological test was strongly positive four days before conclusive hematological changes were demonstrable.

Case 4. Association of the antibodies with a false positive Wassermanan reaction.

W. D., a white, male, student, aged 16, was admitted to the Union Memorial Hospital on May 17, 1933, complaining of stiffness of the neck.

Ten days before admission the patient developed a stiffness of the left side of his neck which was followed soon after by generalized cervical aching. There were night sweats and sore throat for two days preceding entry.

On admission, the temperature was 100.8° F. The cervical, axillary, and inguinal lymph glands were palpable; the left cervical glands were markedly enlarged. The spleen was readily felt. There was a follicular inflammation of tonsillar stumps. The leukocyte count was 10,920 with a differential formula of polymorphonuclears 32 per cent, small lymphocytes 65 per cent, large lymphocytes 3 per cent.

For a week the patient ran moderate fever and suffered from a continued sore throat. Subsequent convalescence was uneventful.

A Wassermann test on May 19 was positive. This finding was verified several times thereafter, until, by July 6, the Wassermann reaction had become completely negative. Heterophile antibodies were present on the first occasion to a titer of 1 to 2,048. The titer gradually declined, as indicated in Table VI, until it fell within normal limits on August 10. By this time, the leukocyte count had fallen to normal, while there was still a slight lymphocytosis of 49 per cent.

The chief feature of interest here was the occurrence of a positive Wassermann reaction in a serum which also contained sheep cell agglutinins. Among others, Parkes Weber (8) has reported the occasional presence of a false positive Wassermann reaction in infectious mononucleosis.

Case 5. No increase in heterophile antibodies in a child of six with infectious mononucleosis.

B. C., a white, male child, aged 6, was admitted to the Sinai Hospital on October 8, 1933, complaining of cough.

This was the twelfth day of an illness characterized by fever and moderate cough. The child did not suffer from sore throat.

On admission the temperature was 100° F. In addition to a general glandular enlargement and a palpable spleen, there were signs of a localized pneumonic process at the right base. The leukocyte count was 12,000; 45 per cent polymorphonuclears, 47 per cent lymphocytes, 8 per cent monocytes. Many of the lymphocytes were of the type characteristic of infectious mononucleosis. Sheep cell agglutinins on October 9, were present in the patient's serum to a titer of 1 to 4.

TABLE VI

Heterophile antibody titer, leukocyte count, differential and titered Wassermann in Case 4

Date	Sheep cell agglutinins										Wassermann reaction	W.B.C.	Lympho-cytes
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048		*per c. mm.*	*per cent*
May 19.........	+++	+++	+++	++	++	++	++	++	+	+	Positive 1 : 4+	17,500	71
May 25.........	+++	+++	++	++	++	+	+	±	-	-	Positive 1 : 150	6,200	86
June 23.........	Not done										Doubtful	6,800	66
July 6.........	+++	++	++	+	±	-	-	-	-	-	Negative	7,080	35
August 10......	+	-	-	-	-	-	-	-	-	-	Negative	5,700	49

The boy recovered rapidly so that he was discharged a week later.

On November 20, 1933, he returned to the Hospital once more with fever and signs of pneumonia, which had reappeared. The glandular enlargement was no longer present while the spleen was barely palpable. Temperature and physical signs were again normal within three days. The leukocyte count on this admission was 18,250; 49 per cent polymorphonuclears, 24 per cent small lymphocytes, 19 per cent large lymphocytes, 8 per cent monocytes. A week after entry his serum gave a plus minus agglutination in a dilution of 1 to 4 with sheep cells, or essentially the same value as was found seven weeks previously.

This was one of the two cases clinically considered infectious mononucleosis, in which heterophile agglutinins were not found to exist above normal limits. The second instance was also a boy of six with characteristic physical and hematological findings. At the height of his course, his serum agglutinated sheep cells to a titer of 1 to 16. Neither of the children, it should be noted, had sore throats at any stage of their illnesses.

Other cases associated with heterophile antibodies

Two other cases of especial interest will now be presented.

Case 16. Probable infectious mononucleosis accompanied by agglutinations with multiple bacterial antigens.

Dr. C. C. B., a white, female member of the House Staff, aged 26, was admitted to the Johns Hopkins Hospital on August 18, 1932, complaining of fever and headache. Two days previously, she had developed headache, pains in the eyes, chills, fever and sweats. Her leukocyte count was found to be 3,000. On the following day, when a rhinitis had appeared, the leukocytes were 3,600.

On admission her temperature was 102° F. She had a slightly red throat, a patch of urticaria in the right axilla and small lymph glands at the angles of the jaw. There were no other palpable nodes and no demonstrable splenic enlargement. The leukocyte count was again 3,000; 65 per cent polymorphonuclears, 30 per cent small lymphocytes, 5 per cent monocytes. Fever persisted for one week. A white blood cell count on the seventh day of the disease was 8,350; another two days later was 9,000; 38 per cent polymorphonuclears, 50 per cent lymphocytes, 12 per cent monocytes. Many of the lymphocytes are described in the record as "young forms." Unfortunately, the smear was not available for subsequent study. Cultures of the blood, stools, urine and throat gave no further information. The patient was discharged on August 24, but felt below par for some time thereafter, being troubled by a mild sore throat and a dry conjunctivitis. The diagnosis was recorded as rhinopharyngitis.

Shortly after admission when the patient's serum was found to agglutinate the paratyphoid B antigen to a titer of 1 to 160, the behavior with other antigens was investigated, with the results indicated in Table VII. It was the clinical impression that the patient did not have paratyphoid fever, although her serum contained agglutinins for *B. paratyphosus B* as well as for several other organisms. Heterophile antibodies were first measured two months after the onset of the disease. At that time their titer was 1 to 256. Subsequently all the agglutinins gradually decreased to approach a normal level.

Several other antigens were tested in addition to those tabulated above. but with negative results. Among these were *B. suipestifer I., B. proteus X–19*, and the three strains of *B. melitensis*.

Whatever interpretation one chooses to apply to these findings, one statement would seem warranted; namely, that the group of heterologous antibodies must have appeared in the course of the acute illness. (The patient had received typhoid vaccine over a year previously.)

Two points worthy of comment emerge from this case. In the first place, it indicates the value of determining sheep cell agglutinins even after a delayed period following the acute stage of a disease, for it was not until

TABLE VII

Values of agglutination titers with various antigens in Case 16

Date	Agglutination titer			
	August 24, 1932	November 11, 1932	January 25, 1933	May 30, 1933
Antigen				
Sheep cells	Not done	1 : 256	1 : 64	1 : 16
B. typhosus	1 : 40	1 : 40	1 : 20	0
B. paratyphosus A	1 : 80	1 : 80	1 : 40	0
B. paratyphosus B	1 : 320	1 : 320	1 : 80	1 : 20
B. aertrycke	0	1 : 160	0	0
B. suipestifer II	1 : 320	1 : 80	1 : 40	1 : 40
B. enteriditis	1 : 160	0	0	0

the heterophile antibody test was found positive two months later, that the history records were scanned for confirmatory evidence which the previously neglected differential count seemed to provide. No definite assertion can be made, but it seems highly probable that the diagnosis should have been infectious mononucleosis.[2] In the second place, here is an instance of human blood serum containing a number of heterologous bacterial antibodies in addition to those against sheep cells.

The frequency of bacterial agglutinins in infectious mononucleosis was investigated. In 14 cases, excluding the present ones, found in the hospital records, one or more agglutinations had been done in six instances. A Widal was found to be negative in all six; melitensis agglutinations negative in two, but a third showed significant findings: an agglutination for the porcine strain of B. melitensis 1 to 640. This was a medical student about whom there was no collateral evidence to suggest Malta fever. After running the typical course of a moderately severe infectious mononucleosis,

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[2] A patient (Case 14, Table IV) subsequently observed, during his acute illness, ran a course similar in essential details to the present one. The serum of this patient, too, agglutinated B. typhosus, paratyphosus B, and suipestifer. At first he was considered to be suffering from typhoid fever, but in the course of the first week of the disease his blood developed the characteristic changes of infectious mononucleosis. During this time, the leukocyte count varied between 3,000 and 5,000. His serum eventually agglutinated sheep red cells in a dilution of 1 to 4,096.

he recovered completely. In five cases of the present group, agglutinations were carried out for *B. typhosus, paratyphosus A* and *B,* as well as for two strains of *B. suipestifer.* For the typhoid group of organisms, agglutinins in 1 to 40 dilution or higher were found in two instances. For the suipestifer group they were found in three, and all of these in 1 to 80 dilution or above. Nothing of note was unearthed in the remaining cases with the exception of the nurse (Case 3) who had recently received typhoid vaccine. Her serum agglutinated the typhoid group in low titer but with *B. suipestifer* gave negative results. Kuttner (9) has observed positive suipestifer agglutinations only in patients from whom the organism has been isolated, and occasionally in other members of the same families when there has been reason to suspect, from the history, that the latter may have recently been afflicted with the same disease. From this one may conclude that, not uncommonly, unrelated bacterial agglutinins are present in infectious mononucleosis.

Multiple bacterial agglutinins were observed more frequently in that form of infectious mononucleosis that started with leukopenia than in the more usual type characterized by leukocytosis. The leukopenic variety is the one in which heterophile antibody determinations are of especial value. In two of the present fifteen cases (Cases 8 and 11, Table IV) pentnucleotide was administered before the true nature of the disease was recognized. In each instance, the white blood cell counts were at first below 5,000, which aroused a suspicion of agranulocytosis.

Case 17. Thrombocytopenic purpura accompanied by heterophile antibodies and a false positive Wassermann reaction.

I. W., a white, female student, aged 16, was admitted to the Johns Hopkins Hospital on March 7, 1933, complaining of nose-bleeds. There was a history of some familial hemorrhagic diathesis. Starting at the age of six years the patient had suffered episodes of bleeding from various sites. Six days before entry a spontaneous epistaxis set in. Following packing of the nose a pharyngitis and otitis media appeared.

On admission, there were the typical findings of purpura hemorrhagica complicated by a draining right ear: fever, purpuric lesions on the skin, a palpable liver and spleen. At no time was there generalized adenopathy or jaundice. Examination of the blood showed a mild secondary anemia, a leukocyte count of 10,000, 54 per cent polymorphonuclears, 11 per cent large lymphocytes, 29 per cent small lymphocytes, 6 per cent monocytes. The platelet count was 70,000; bleeding time 25 minutes; clotting time normal. Coincident with a transfusion the bleeding gradually subsided so that the patient was ready for discharge one month later. Within three weeks the bleeding time had become normal although the platelets continued about 100,000. Subsequent differential counts were not noteworthy.

A Wassermann reaction on the day of admission was positive while a flocculation test, after the Eagle technique (10), was negative. False positive Wassermann reactions are known to occur occasionally in this disease. Three days later heterophile agglutinins were first sought for and found to exist up to a titer of 1 to 1,024. Subsequent findings are outlined in Table VIII.

TABLE VIII

Relation of heterophile antibodies to titered Wassermann reactions in a case of purpura hemorrhagica (Case 17)

Date	Sheep cell agglutinins									Wassermann
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	titered
1933										
March 7	Not done									Positive 1 : 4+
March 10	+++	+++	+++	++	++	++	+	±	±	Positive 1 : 4+
March 16	Not done									Positive 1 : 150
March 20	+++	+++	++	++	++	++	+	±	—	Not done
March 24	+++	+++	+++	++	++	++	+	—	—	Negative
March 28	+++	+++	++	++	+	±	—	—	—	Anticomplimentary
April 6	+++	+++	++	++	±	—	—	—	—	Negative
April 26	++	++	+	—	—	—	—	—	—	Not done
August 2	—	—	—	—	—	—	—	—	—	Negative

Note: Flocculation test always negative.

The only moot point is whether infectious mononucleosis co-existed with the purpura. The findings were all compatible with the single disease, purpura, from which the patient unquestionably suffered, but the possibility of an atypical form of infectious mononucleosis can not be dismissed.

The nature of the false positive Wassermann reaction in infectious mononucleosis

It has been possible to analyze the character of the false positive Wassermann reaction which occurred in the one case of infectious mononucleosis and in the instance of purpura. The term " false positive " is used to denote the situation wherein the Wassermann reaction becomes transiently positive in the absence of syphilitic infection. One type of such reaction— termed by Eagle the " anomalous false positive reaction "—was observed in these two cases when the complement fixation test was temporarily positive, while a flocculation test, after the Eagle modification, was consistently negative.

That two diseases, unrelated so far as prevailing knowledge reveals, both of which on occasions are known to be associated with false positive Wassermann reactions, also may present heterophile antibodies, would seem to be more than a coincidence. It appears that the positive Wassermann reaction exists *in spite of* the antibodies, for it is apparent that sheep cell hemolysins in serum would upset the hemolytic system in the Wassermann set-up and tend to make a positive reaction negative. Titered Wassermann reactions carried out on serum from the case of infectious mononucleosis, before and after absorbing the hemolysins with sheep cells, supported this conclusion. Starting with 0.2 cc. of serum the values for successive dilutions were 2–2–4–4–4–4–2 and 4–4–4–4–4–4–2 respectively;

more strongly positive in the lower dilutions, therefore, after the removal of the antibodies. (The degree of positivity is represented as ranging from 1 up to 4.)

Heterophile antibodies were found lacking in the sera of several patients who, without any stigmata of syphilis, had intermittently positive Wassermann reactions but negative flocculation tests. Again, the antibodies were not demonstrated in some of the other conditions in which false positive Wassermann reactions are reputed to occur, such as jaundice, pneumonia and scarlet fever.

DISCUSSION

The data presented herein relative to the occurrence of heterophile antibodies in infectious mononucleosis are in accord, in most respects, with the original findings of Paul and Bunnell (1), and the subsequent report of Bunnell (4). In a variety of clinical conditions, sheep cell agglutinins were found to be present in the patients' sera in concentrations now recognized as falling within normal limits. In thirteen out of fifteen cases of infectious mononucleosis, the agglutinin titers of the blood sera were elevated. Two instances of the disease, however, gave perfectly normal figures in so far as sheep cell agglutinins were concerned.

Rosenthal and Wenkebach (2) recounted the histories of ten patients in whom the only detail whereby their illnesses could be differentiated from infectious mononucleosis was the normal concentration of heterophile agglutinins in their blood sera. On the basis of this finding they suggested that in those instances where the agglutination test was positive, the patient had infectious mononucleosis, while in those where the test was negative, the disease process was glandular fever. Such an interpretation must be cautiously made, however, both because the cause of the increase of heterophile antibody is unknown and because of the wide range of antibody concentration in normal people. One must remember that if the increased titer of agglutinins in infectious mononucleosis merely represents an enhancement of the concentration of antibodies already present, then there is just as much increase in a patient's serum with a normal agglutination titer of 1 to 1, rising to 1 to 16 as there would be in one starting at 1 to 8 and rising to 1 to 128. Yet the former would be considered a negative heterophile antibody test and the latter a positive one. In this sense, no such sharp distinction can be made here as is possible between a positive and negative Wassermann reaction.

The phenomenon of heterophile antibody production in infectious mononucleosis may indicate some valuable guides pointing towards the etiology of the disease. After the injection of horse serum, which contains the heterophile antigen, the appearance of the antibodies is comprehensible. The source of the antigen in infectious mononucleosis may be extrinsic or intrinsic. If the former, it must be found in the organism or virus which

causes the malady; if the latter, it must be associated with the tissues of the patient. Paul and Bunnell were unable to find supportive evidence to incriminate Vincent's fusiform bacilli as the offenders. In connection with their experiments, it is pertinent to recall the fact, that although the dysentery Shiga bacillus contains heterophile antigen, two human carriers of this organism did not possess sheep cell agglutinins in more than normal concentrations.

Of all the human tissues that have been tested, only the red cells from type A subjects (Moss Groups I and II), seem to contain heterophile antigen. Normally, such persons do not show a significantly higher titer of sheep cell agglutinins than the other two groups. One might hypothecate that in infectious mononucleosis red cells are, for some reason, broken down with the liberation of the antigen which subsequently produces the antibodies. There is ample evidence that such is not the case. Sheep cell agglutinins appeared in Moss Group IV patients with just as great regularity as in Group II. Furthermore, several patients receiving multiple transfusions with Group II blood did not develop an increased antibody titer.

It is plain that the substance responsible for the positive Wassermann reaction in infectious mononucleosis and purpura hemorrhagica is not reagin, which appears in the serum of syphilitics. If it were, the flocculation test should be positive as well as the complement fixation reaction. Nor is it the agent which agglutinates sheep cells. While this latter substance is present in infectious mononucleosis with greater consistency than either the complement-fixing material for the Wassermann reaction or the various bacterial agglutinins, the significance of the appearance of these three agents would seem to be the same: i.e., evidence of the versatility of antibody responses in infectious mononucleosis.

CONCLUSIONS

Blood sera of 300 adult hospital patients have been tested for the presence of agglutinins for sheep's red cells. In this control group the agglutinin titer never exceeded 1 to 16. Thirteen out of fifteen accepted cases of infectious mononucleosis, studied in the acute stage of the disease, showed an increased titer of agglutinins for sheep's red cells attaining dilutions as high as 1 to 4,096. Except in these subjects, titers above normal levels were encountered only under the following circumstances: (1) in individuals who had received injections of horse serum, which contains heterophile antigen; (2) in one patient whose serum contained agglutinins for several bacterial antigens, the exact nature of whose disease was never definitely determined, but who probably suffered from infectious mononucleosis; (3) in a patient with purpura hemorrhagica who presented scant evidence of a concomitant infectious mononucleosis. The blood serum from several cases of infectious mononucleosis contained agglutinins for a num-

ber of bacterial antigens. However, in none of the diseases in which bacterial agglutinations occur were heterophile antibodies unusually increased. Similar observations were made in a study of a number of the blood diseases. One case of infectious mononucleosis is detailed in which the Wassermann reaction became temporarily positive. A similar state of affairs was met with in a patient with purpura hemorrhagica. Of the other clinical conditions in which a false positive Wassermann may occur, none with an increased heterophile antibody titer was encountered.

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rabbit in 18 to 36 hours. Save for these exceptions mentioned, the methods of study paralleled those of Dennis.

Four types of organisms were used, *B. pyocyaneus*, *Streptococcus hemolyticus*, *Streptococcus viridans* and *Staphylococcus aureus*. Two different strains of *Streptococcus viridans* from two cases of subacute bacterial endocarditis were used for different animals, and five different strains of *Staphylococcus aureus*, including one cultured directly from the fresh pus of a furuncle, were used successively.

Parchment capsules were made by cutting down two Schleicher and Schüll diffusion shells No. 579–A (16×50 mm.) to about 2.5 cm. in length and fitting them together. Seventy per cent alcohol was used for sterilizing the shells before they were fitted. The joint of each capsule was sealed with water hardened celloidin. Five cc.[2] of broth infusion or serum broth cultures of organisms, 18 to 24 hours old, were put into the capsule through one end with a sterile needle and syringe. The small hole thus made was sealed with celloidin. The capsule was then well washed in 70 per cent alcohol before it was placed in the abdominal cavity. The operation was a simple midline incision 3 to 4 cm. long into the peritoneal cavity, using strict aseptic precautions. After the capsule had been inserted, the wound was carefully sutured in the usual manner.

Healthy, mature rabbits without anemia were used in all experiments. Occasionally a rabbit had, at different times, more than one capsule containing microorganisms; for if weeks or months after one experiment the rabbit's condition was good, another capsule containing a culture was placed in the abdominal cavity and the rabbit was given a new number. These instances can be noted in Table I for the new number was always made by adding a numeral to the previous number. Thus rabbit Number 10 became 10–1, Rabbit 1A became 1A2, etc.

For control purposes and for determination of a stabilization level, blood counts were done, usually daily, for periods of 1 to 16 days before operation. Red cell counts and hemoglobin determinations were made once or usually more times on each rabbit before, and when possible, after the experiments. After the capsule was in place, white cell counts were done each morning or when indicated, more frequently, until the animal died or until such time as it was deemed no longer necessary. Daily postoperative leukocyte counts were always done for 7 days or more when the rabbits lived, and usually three times a week for several weeks longer. All counts were done by the usual methods using pipettes and a hemacytometer certified by the United States Bureau of Standards. All leukocyte counts were done in duplicate, two pipettes being filled for each count. As a count from each pipette was made on both sides of the hemacytometer, each count reported here represents the average of four counts, thus insuring greater accuracy. The pipettes were numbered so that the same two were used each day for a given animal throughout the period of observation. Differential counts were made on cover slip smears stained with Wright's stain and in each instance the percentage of cells was derived from a count of 300 cells. Since interest is chiefly attached to the neutrophiles in these experiments, and in order to conserve space, only the percentage figures for these cells with the total counts are given, though complete differential counts with the usual division of cells were always made.

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RESULTS

The results are tabulated in Table I. In reporting the total and differential counts, fractions are omitted and the closest round figure given. For example, 7775 white blood cells are reported as 7800, and 49.3 per cent neutrophiles as 49 per cent.

Examination of the results clearly shows a failure to produce distinct leukopenia of significant degree or duration except in two animals, Rabbits B and 5B2, with *B. pyocyaneus.* Though the total neutrophile count in the peripheral blood decreased in the two instances where leukopenia occurred, there was failure to produce distinct relative granulopenia in a single instance. In the case of Rabbit 5B2, a culture was made from outside of the capsule as soon as the abdomen was opened after death and this showed *B. pyocyaneus,* indicating that the capsule was not completely sealed. Unfortunately, this same procedure was not carried out earlier when Rabbit B died. It should be noted in this connection, however, that subsequent injections of pure cultures of *B. pyocyaneus* in varying doses, directly into the peritoneal cavity, were not efficacious in producing leukopenia.

Postmortem examination and careful examination of the capsule was done in every rabbit that died. In three instances the capsule was definitely not intact, in the others the capsule was intact and usually deeply imbedded in thick white pus.

Many of the neutrophiles, in the two cases where leukopenia and prompt death (within 40 hours) resulted after *B. pyocyaneus* implantation, were large and degenerated at the time the leukocyte count was falling. These cells closely resembled the cells shown in the photographs of Dennis' paper. The bone marrow of these cases was, however, normal in appearance or hyperplastic. There was no sign of degeneration of the cells as noted by Dennis.

The experiments using *Staphylococcus aureus, Streptococcus hemolyticus* and *Streptococcus viridans* usually resulted in leukocytosis and a higher level of neutrophile cells for several days with gradual return to the preoperative level, or else a persistent, or sometimes progressive. leukocytosis. In a few experiments, slight temporary decrease in the number of leukocytes occurred, associated, except in two instances, with an increase in percentage of neutrophiles.

DISCUSSION

Clearly the results reported here will not confirm the conclusion of Dennis that encapsulated pyogenic bacteria, acting as a focus, produce granulopenia. It is appreciated that identical strains of organisms were not available. Because of our negative findings, the results of Dennis (3) were subjected to close analysis. When these are studied, the results with *Streptococcus viridans* alone seem to be very significant. Thus in Table I

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TABLE I

Experimental data

Date	Culture of organisms	Rabbit number	Preoperative period	Average preoperative W.B.C. and percentage of neutrophiles	Results
1933 March 8	*Staph. aureus-A,* B.B.I. 5 cc.	1A	days 7	13,100—26	Count fell to 6900; N. 40% the 1st P.O. day. Subsequent rise of W.B.C. to pre. op. level. N, 33 to 50%. Followed 10 weeks P.O. Used again as 1A2
	Staph. aureus-A, B.B.I. 5 cc.	2A	7	12,300—51	Lowest P.O. count 12,100, day after operation. Progressive increase to 54,700 on 15th P.O. day. Death on 20th P.O. day; count 35,400. P.M. showed many large abscesses in liver. Capsule apparently intact
	Control sterile, B.B.I. 5 cc.	3A	7	13,500—46	Lowest P.O. count 12,900, day after operation. Rose to 22,000 on 4th P.O. day. Death 16th P.O. day. P.M. showed no cause for death. Capsule apparently intact, partially walled off
March 30	Control sterile, B.B.I. 5 cc.	1B	4	10,800—46	Lowest count, 8700, day after operation. N, 78%. Animal found dead a.m. of 2nd P.O. day. P.M. showed peritonitis
	Staph. aureus No. 156, B.B.I. 5 cc.	2B	4	9600—42	Count 1st P.O. day, 18,500. N, 80%. Lowest count 43rd P.O. day, 10,700; N, 47. Used again as 2B2
	Staph. aureus No. 157, B.B.I. 5 cc.	4B	3	7500—39	Count lowest 1st P.O. day, 6800; N, 58%. A.M. of day of operation count was 6500; N, 38%. Progressive increase in W.B.C. thereafter to 7th P.O. day. Count on 26th P.O. day 12,200; N, 45%. Rabbit found dead on 39th P.O. day. P.M. showed intestinal obstruction. Capsule intact, well walled off
	Staph. aureus No. 157, B.B.I. 5 cc.	5B	3	10,400—51	Count lowest 1st P.O. day, 4900; N, 72%. Progressive increase thereafter to 30,000 on 20th P.O. day, then lower. Used again as 5B2

TABLE I (continued)

Date	Culture of organisms	Rabbit number	Pre-operative period	Average preoperative W.B.C. and percentage of neutrophiles	Results
			days		
1933 April 19	Staph. aureus (from furuncle) B.B.I. 5 cc.	7B	16	13,000—48	Count lowest 2nd P.O. day, 9600; N, 44%. Thereafter above control level to 16th P.O. day. Used again as 7B2
	Staph. aureus (from furuncle) B.B.I. 5 cc.	8B	16	13,300—39	Count lowest 1st P.O. day, 9300; N, 73%. On 3rd P.O. day, 9750; N, 24%. Thereafter, progressive increase to 7th P.O. day. Count on 16th P.O. day, 14,700; N, 51%
May 16	B. pyocyaneus, B.B.I. 5 cc.	A	6	9200—47	Count lowest 1st P.O. day, 6300; N, 66%. Count higher thereafter and except for one day, above preoperative level to 53rd P.O. day. Used again as A1
	B. pyocyaneus, B.B.I. 5 cc.	B	6	9200—40	1st P.O. day: 10 a.m. 2300; N, 43%; 3:15 p.m.1850; N, 29%; 4:30 p.m. 2800; N, 30% Many of the neutrophiles in smear large and degenerated. Immediately after this count, rabbit died. P.M. showed capsule intact, no gross lesion. Microscopic examination of bone marrow showed no abnormality
May 19	B. pyocyaneus, B.B.I. 2 cc.	C	5	7900—34	Lowest count 2nd P.O. day, 6900; N, 41%. Counts higher thereafter. Last count 21st P.O. day, 12,900; N, 51%
	B. pyocyaneus, B.B.I. 5 cc.	D	5	7800—44	Lowest count 1st P.O. day, 7800; N, 72%. Counts higher thereafter. Count on 20th P.O. day, 10,800; N, 40%

TABLE I (continued)

Date	Culture of organisms	Rabbit number	Preoperative period	Average preoperative W.B.C. and percentage of neutrophiles	Results
1933 May 31	*B. pyocyaneus,* B.B.I. 6 cc.	5B2	*days* 1	19,400—48	Count 1st P.O. day at 11 a.m., 6700; N, 86%. 4 p.m. 4700; N, 79%; 8:45 p.m. 4200; N, 66%. Neutrophiles, many large and degenerated. Rabbit dead next a.m. P.M. showed extensive pneumonia of right lung. Bone marrow congested, slight hyperplasia. Capsule appeared to be intact but culture from about it showed *B. pyocyaneus*
	B. pyocyaneus, B.B.I. 6 cc.	7B2	1	16,500—41	Lowest count 5th P.O. day, 10,100; N, 57%. Then rise to 21st P.O. day. Sudden fall 22nd P.O. day to 9900; N, 68%. Neutrophiles not degenerated. Rabbit dead 23rd P.O. day. P.M. showed multiple pulmonary infarcts and abscesses. Dilated right auricle. Bone marrow pale. Microscopic examination showed leukocytic hyperplasia
May 31	*Strep. hemolyticus,* B.B.I. 6 cc.	1A2	1	11,900—39	Lowest count 3rd P.O. day, 7900; N, 45%. Thereafter counts higher. Highest, 10,200; N, 38%; 13th P.O. day
	Strep. hemolyticus, B.B.I. 5 cc.	2B2	1	10,400—42	1st P.O. day 9400; N, 62%. Higher counts thereafter. Rabbit used for another experiment later
June 9	*Strep. hemolyticus,* B.B.I. 6 cc.	E	2	10,100—52	Lowest count 11,500; N, 61% 1st P.O. day. Progressive increase to 17,400; N, 60%; on 6th P.O. day. Then to preoperative level by 30th P.O. day
	Strep. hemolyticus, B.B.I. 5 cc.	F	2	17,800—45	Lowest count 7th P.O. day, 11,400; N, 53%. Other counts high. Last, 18th P.O. day, 15,300; N, 43%. Rabbit died 26th P.O. day. P.M. showed capsule intact, adhesions about

TABLE I (*continued*)

Date	Culture of organisms	Rabbit number	Preoperative period	Average preoperative W.B.C. and percentage of neutrophiles	Results
			days		
1955 September 27	Strep. viridans M from subacute bacterial endocarditis, S.B. 5 cc.	A1	3	12,300—38	Lowest count, 1st P.O. day, 11,700; N, 72%. Higher thereafter, maximum 20,700; N, 56%, 3rd P.O. day. Above preoperative level as long as followed, 16 days
	Strep. viridans M from subacute bacterial endocarditis, S.B. 5 cc.	10	3	7600—35	1st P.O. day, 9700; N, 69%. No leukopenia developed. Used again as 10-1
	Strep. viridans W from subacute bacterial endocarditis, S.B. 5 cc.	11	3	9800—35	Lowest count, 3rd P.O. day, 8000; N, 49%. Above preoperative level thereafter. Used again as 11-1
October 18	Strep. viridans W, S.B. 5 cc.	10-1	2	14,700—61	1st P.O. day, 13,800; N, 51%. Rabbit had diarrhea. Found dead 2nd P.O. day. P.M. showed pneumonia, right lower lobe. Capsule appeared intact but culture from within showed few strep, many gram-negative rods
	Strep. viridans W, S.B. 5 cc.	11-1	2	13,700—31	Count lowest 2nd P.O. day, 9000; N, 39%. Higher thereafter. Leukopenia did not occur

TABLE I (*continued*)

Date	Culture of organisms	Rabbit number	Pre-operative period	Average preoperative W.B.C. and percentage of neutrophiles	Results
			days		
1933 November 15	*Staph. aureus* C. (after passage through 3 rabbits) S.B. 5 cc.	13	2	13,900—35	Count rose to 28,000; N, 80%, 1st P.O. day. Remained high. Rabbit seemed to have a cold. Last count 7th P.O. day, 18,800; N, 43%. Rabbit found dead 12th P.O. day. P.M. showed patchy pneumonia, left lung. Capsule intact, imbedded in caseous white pus
	Staph. aureus C. (after passage through 3 rabbits) S.B. 5 cc.	14	2	16,900—59	Lowest count, 3rd P.O. day, 10,300; N, 60%. Counts higher thereafter. 18,800; N, 47%, 7th P.O. day. Count 55th day P.O., 17,400; N, 66%.

Key to Abbreviations:

B.B.I.—Beef broth infusion.
S.B.—Serum broth.
P.O.—Postoperative.
P.M.—Postmortem.
N.—Neutrophile.

(3), Rabbit 9–9, for example, the changes in the total and differential counts do not appear significant when the well known fluctuations in rabbits are taken into account. On the 32d postoperative day the total and differential leukocyte counts resemble those of the day of operation, and on the third day before death, when the white cell count was 11,400, there were 20 per cent neutrophiles and 30 per cent band forms, whereas on the day of operation the two forms also totalled 50 per cent with 47 per cent neutrophiles. Table II also records the results of an experiment after *Staphylococcus aureus*. Here a progressive increase in white cells from 8550 to 29,850 occurred. The percentage of cells of the granular series excluding eosinophiles and basophiles, fell from 74 per cent to 23 per cent, the day before death, but the total number of these cells per cu. mm. in the peripheral blood on this day was 6866 as compared to 6327 the day of operation. Similarly, in Tables III and IV after *Streptococcus hemolyticus*, the results do not appear to be significant. In the latter experiment leukopenia occurred but the percentage of granular cells at this time was higher than immediately after the operation. Unfortunately the author does not give any of the preoperative counts. The results after *Streptococcus viridans*, Tables V and VI, are striking however and of great interest. Our failure to produce similar results with *Streptococcus viridans* is not explicable. The rapid development of leukopenia resembles that obtained after *B. pyocyaneus* reported here except that with this organism, significant relative neutropenia did not occur.

Attention might be directed to the control experiment of Table VII of Dennis' paper as this shows a distinct fall in the white count and neutrophiles on the fourth day as compared to the count of the first day. However, this original reported count is postoperative and since the preoperative counts are not given, proper evaluation of the results is not possible.

CONCLUSIONS

1. An attempt to produce leukopenia or granulocytopenia in rabbits by the introduction into the abdomen of encapsulated cultures of various pyogenic bacteria was unsuccessful except in the case of *B. pyocyaneus*. With this organism there was produced an acute leukopenia and death (within 40 hours) without significant decrease in the percentage of neutrophiles.

2. In none of the 26 experiments upon 19 rabbits was it possible to produce a blood picture resembling clinical benign or malignant neutropenia.

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THE EFFECT OF POSTURE (STANDING) ON THE SERUM PROTEIN CONCENTRATION AND COLLOID OSMOTIC PRESSURE OF BLOOD FROM THE FOOT IN RELATION TO THE FORMATION OF EDEMA

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These studies were made to determine the effect of the erect posture on the colloid osmotic pressure and the concentration of the serum proteins of the blood of the foot with particular reference to the occurrence of edema in persons with slightly lowered serum proteins (1, 4). Studies by others (2, 9, 10, 11) have shown that standing causes a general concentration of blood in the body, but determinations of the serum protein concentration and osmotic pressure of blood from the local dependent parts have not been reported.

EXPERIMENTAL

The subjects included normal persons, five patients with nutritional edema and one patient with nephrosis. Four of the cases of nutritional edema were of the simple, endemic type described previously (1, 4); in one case the nutritional edema was associated with a carcinoma of the stomach. In some of the patients the edema was slight.

The following procedure was employed. One or two hours after a light breakfast the subject reclined for an hour or more. At the end of this time the venous pressure in the arm was determined by the direct method and a sample of blood was drawn for the determination of the serum protein concentration, colloid osmotic pressure and in some experiments, the relative cell volume (hematocrit). The volume of the leg while standing was then roughly determined by measuring the amount of water it displaced from a rigid metal boot which reached to approximately the mid-thigh. The subject now stood, moving as little as possible, at an angle of 75 degrees [1] against an inclined table. A supporting belt under the arms and across the knees, so arranged as to avoid constriction, proved a necessary precaution. At the end of an hour or more the pressure in a

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[1] According to Turner, Newton and Haynes (Am. J. Physiol., 1930, 94, 507) this position is equivalent to the vertical in respect to hydrostatic and vascular changes.

vein of the foot, on the dorsum or at the level of the internal malleolus, was measured and a sample of blood was drawn from the same vein for the determinations listed above. In several experiments a sample of blood was also drawn from the arm for comparison with that from the foot. As soon as possible after the " standing " specimens were obtained the volume of the leg was measured again. In one experiment the order was reversed and the subject first stood and then reclined. Various technical difficulties and, in some instances, fainting on the part of the subject, made it impossible to carry out these procedures in strictly uniform time. In some experiments fainting made it necessary for the subject to recline for a short time after the standing period before blood could be obtained from the arm or the leg volume measured. These variations may have caused some irregularities in the results. Most of the experiments were purposely carried out during hot weather. The serum protein concentration was determined by the method of Howe (3) as described elsewhere (4). The colloid osmotic pressure was measured by Krogh's method (5) as modified by Wells (6). Hematocrit determinations were carried out in duplicate, using tubes with an inside diameter of 1 mm. and a length of 10 cm. The filled tubes were rotated at a speed of 3000 r.p.m. for 30 minutes. Heparin was used as the anticoagulant.

<div align="center">RESULTS</div>

The principal data are shown in the accompanying tables and charts. All but two of the normal subjects fainted during or at the end of the standing period and those two nearly lost consciousness. In most instances fainting seemed to be precipitated by the venipuncture in the foot but pallor of the face, yawning and giddiness before the venipuncture were indicative of an increasing cerebral anemia. One normal subject became nauseated and vomited. None of the *patients* lost consciousness but two became faint and giddy. Two of the patients voided large amounts of urine, 800 and 720 cc., respectively, at the end of the period of reclining, though the bladder had been emptied shortly before lying down. This fluid was partly replaced by drinking water.

The data show very clearly that standing for approximately one hour causes a large increase in the concentration of serum proteins and a great rise in the osmotic pressure of blood in the feet and legs of normal subjects.[2] (Table I.) Thus, in Subject 1, who stood for 62 minutes with a

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[2] It is assumed that blood in the foot at the end of the reclining period had the same concentration of protein and the same colloid osmotic pressure as blood from the arm. With the subject in the reclining position it is very difficult and many times impossible to obtain a sufficiently large sample of blood from the foot without using stasis. In a special study practically identical values were found in blood from these two regions after the subjects had reclined an hour or more. The correspondence is probably not as close in patients with edema due to a slower and irregular resorption of fluid from the tissues of the feet and legs.

TABLE I

Experimental data

Subject	Serum protein									Colloid osmotic pressure			Venous pressure	Hematocrit cell volume		Increase in leg volume		Standing time	Pitting edema (legs)
	Total		Albumin			Globulin													
		Difference grams per cent	Increase per cent		Difference grams per cent	Increase per cent		Difference grams per cent	Increase per cent	cm. water	Difference cm. water	Increase per cent	cm. water	per cent	Difference per cent	cc.	cc. per 100 cc. leg	minutes	
Normals	grams per cent			grams per cent			grams per cent												
1 R*	7.14	1.91	26.7	4.79	1.29	26.9	2.35	0.62	26.3	37.1	14.8	39.8	9.7	40.0	6.0	260	4.28	62	0
S	9.05			6.08			2.97			51.9			92.7	46.0					
2 R	6.87	2.77	40.3	4.72	1.90	40.2	2.15	0.87	40.4	34.7	22.4	64.5	7.5	37.0	12.0	270	4.63	63	0
S	9.64			6.62			3.02			57.1			109.3	49.0					
3† R	6.69	1.97	29.4	4.57	1.36	29.7	2.12	0.61	28.8	32.9	15.8	48.0	7.4	39.5	5.5	290	4.87	43	0
S	8.66			5.93			2.73			48.7			103.7	45.0					
4 R	7.03	1.27	18.0	4.87	0.73	17.1	2.25	0.45	20.0	34.5	11.0	31.9	11.8	37.0	(clotted)	225	3.11	66	0
S	8.30			5.60			2.70			45.5			96.6	37.0					
5 R	6.33	1.37	21.6	4.13	0.89	21.5	2.20	0.48	21.8	29.8	8.6	29.2	5.7	45.0	15.0	300	3.61	90	+
S	7.70			5.02			2.86			38.5			120.0	60.0					
Average			27.2			27.1			27.5			42.7				272	4.10	66	

TABLE I (continued)

Subject	Posture	Total serum protein (g%)	Total diff (g%)	Total incr (%)	Albumin (g%)	Albumin diff (g%)	Albumin incr (%)	Globulin (g%)	Globulin diff (g%)	Globulin incr (%)	Colloid osmotic (cm water)	Colloid diff (cm water)	Colloid incr (%)	Venous pressure (cm water)	Hematocrit (%)	Hematocrit diff (%)	Leg vol incr (cc)	Leg vol incr (cc/100 cc)	Standing time (min)	Pitting edema (legs)
Patients																				
6	R	6.47	1.45	22.4	4.36	1.09	25.0	2.11	0.36	17.1	31.5	12.2	38.7	4.5	29.3	8.7				+
	S	7.92			5.45			2.47			43.7			105.7	38.0		410	7.45	88	++
7	R	6.58	0.96	14.6	3.62	0.60	16.5	2.96	0.36	12.2	28.8	8.3	28.8	5.2	34.0	4.0				++
	S	7.54			4.22			3.32			37.1			98.6	38.0		250	4.29	75	+++
8	R	6.67	1.87	28.0	4.27	1.22	28.5	2.40	0.65	27.0	31.8	14.9	46.8	10.7						+
	S	8.54			5.49			3.05			46.7			99.5			253	3.07	110	++
9‡	R	4.88	1.39	28.5	3.11	0.83	26.7	1.77	0.56	31.6	19.9	7.4	37.1	1.1						++
	S	6.27			3.94			2.33			27.3			88.4					85	+++
10	R	6.53	2.15	32.9	4.63	1.51	32.6	1.90	0.64	33.6	33.4	16.0	47.9	7.4	34.0	1.5				+
	S	8.68			6.14			2.54			49.4			65.7	35.5		410	7.20	90	++
11§	R	4.16	−.07	−1.7||	2.41	−.13		1.75||		−5.7||	14.4	1.4		7.9						++++
	S	4.09			2.28			1.81			15.8			40.1			330 330	3.79 5.16	85 88	+++++
Average				25.3			25.9			24.3										

* R = Reclining; S = Standing.—Analyses of the edema fluid from Subjects 9 and 11 showed a protein content of 0.37 and 0.27 gram per cent respectively.

† Could not stand continuously.

‡ Reverse order.

§ Sat in chair.

|| Apparent decrease.

venous pressure in the foot of 92.7 cm. of water, the total proteins increased from 7.14 to 9.05 grams per 100 cc. or 26.7 per cent, albumin from 4.79 to 6.08 or 26.9 per cent and globulin from 2.35 to 2.97 or 26.3 per cent. The colloid osmotic pressure rose from 37.1 to 51.9 cm. of water, an increase of 39.8 per cent and the volume of the leg increased 260 cc. or 4.28 cc. per 100 cc. of leg.

Among the normal subjects, venous pressures while standing varied between 92.7 and 120 cm. of water. Standing time was from 43 to 90 minutes. The increase in total protein ranged from 18 to 40 per cent, albumin from 17 to 40 per cent and globulin from 20 to 40 per cent. The average increase in these three values was 27.2, 26.6, and 27.6 per cent respectively. Accompanying the increased concentration of proteins there was a rise in the colloid osmotic pressure of the blood serum ranging from 8.6 to 22.4 cm. of water, an increase of from 29.2 to 64.5 per cent. The average increase was 42.7 per cent. The rise in osmotic pressure was proportionately greater than the increase in the concentration of proteins because of an increase in specific osmotic pressure, i.e., pressure per gram of protein, which becomes greater as the concentration of protein increases (Table II). In one sub-

TABLE II

Increase in specific osmotic pressure (Pressure per gram per cent of protein) with increased concentration of serum proteins

Subject	Reclining	Standing	Difference	Increase
Normals	*cm. water*	*cm. water*	*cm. water*	*per cent*
1	5.2	5.7	0.5	9.6
2	5.1	5.9	0.8	15.7
3	4.9	5.6	0.7	14.2
4	4.9	5.5	0.6	12.2
5	4.7	5.0	0.3	6.4
Average	5.0	5.5	0.5	8.9
Patients				
6	4.9	5.5	0.6	12.2
7	4.4	4.9	0.5	11.3
8	4.8	5.5	0.7	14.6
9	4.1	4.4	0.3	7.3
10	5.1	5.7	0.6	11.8
11	3.5	3.9	0.9	11.5
Average	4.5	5.0	0.5	11.5

ject this increase in specific colloid osmotic pressure amounted to as much as 16 per cent.

With the loss of fluid from the blood there was a corresponding increase in leg volume ranging from 225 to 300 cc. Calculated on a comparable basis of the increase per 100 cc. of leg, the increase ranged from

3.11 to 4.87 cc. None of the strictly normal subjects developed pitting edema. Subject 5, though included in the group of normals, was found to have a serum protein concentration slightly below the lower normal limit and developed slight pitting edema of the legs. Although she stood longer than the other normal subjects pitting edema was already present at the end of 60 minutes.

The concentration of blood was greater in the feet and legs than in the upper part of the body (arms) where the average increase in total protein was only 13.2 per cent, and in osmotic pressure 23.1 per cent, compared with 27.2 and 42.7 per cent respectively in the foot (Table III).

TABLE III

Comparison of the increase in the concentration of serum proteins and in the colloid osmotic pressure of blood in the arm and in the foot after standing

Subject	Total serum protein				Colloid osmotic pressure			
	Re-clining	Stand-ing	Differ-ence	In-crease	Re-clining	Stand-ing	Differ-ence	In-crease
	grams per cent	grams per cent	grams per cent	per cent	cm. water	cm. water	cm. water	per cent
1 Arm..........	7.14	7.82	0.68	9.5	37.1	42.4	5.3	14.2
Leg*..........	7.14	9.05	1.91	26.7	37.1	51.9	14.8	39.8
2 Arm..........	6.87	7.96	1.09	15.8	34.7	43.0	8.3	23.9
Leg..........	6.87	9.64	2.77	40.3	34.7	57.1	22.4	64.5
3 Arm..........	6.69	7.55	0.86	12.8	32.9	40.0	7.1	21.5
Leg..........	6.69	8.66	1.97	29.4	32.9	48.7	15.8	48.0
4 Arm..........	7.03	7.72	0.65	9.2	34.5	44.7	10.2	29.6
Leg..........	7.03	8.30	1.27	18.1	34.5	45.5	11.0	31.9
5 Arm..........	6.33	7.50	1.17	18.5	29.8	37.6	7.8	26.2
Leg..........	6.33	7.70	1.37	21.6	29.8	38.5	8.7	29.2
Average Arm..........				13.2				23.1
Leg..........				27.2				42.7

* The concentration of protein and the colloid osmotic pressure of the blood in the foot is assumed to be the same as in the blood from the arm while the subject was reclining. See footnote 2.

Some of the subjects with edema showed a concentration of serum proteins and a rise of colloid osmotic pressure equal to that observed in some of the normal subjects but the average for the group was somewhat less (Table I). In the case of Subject 7, the increase was less than in any of the normal subjects. Subject 11, with nephrosis, who was unable to stand and sat in a chair with a venous pressure in the foot of 65.7 cm. of water, showed slight apparent decreases in the concentration of total proteins

and albumin which are probably within the range of experimental error. Excluding this latter experiment the increase in total proteins in the subjects with edema ranged from 14.6 to 32.9 per cent, albumin from 16.5 to 32.6 per cent and globulin from 12.2 to 33.6 per cent, an average of 25.3, 25.9 and 24.3 per cent respectively. Colloid osmotic pressure increased from 28.8 to 47.9 per cent. Specific colloid osmotic pressure increased as in the normal subjects and to about the same degree (Table II). The average increase in leg volume, 330 cc., was somewhat greater than in the normal subjects. The increase per 100 cc. of leg ranged from 3.07 to 7.45 cc. with an average of 5.68. The average standing time, however, was considerably longer in the case of the patients. All of the patients had more or less pitting edema which became demonstrably greater on standing.

In those experiments in which hematocrit determinations were made the increase in cell volume after standing indicates that a true concentration of the blood had occurred (Table I). The lack of agreement in the degree of concentration as expressed by the hematocrit readings and the protein content respectively is not due to errors in analysis but to the fact, as Landis and his associates have pointed out (7), that changes in cell and plasma volume are calculated on the basis of whole blood while protein concentration is expressed on the basis of grams per 100 cc. of plasma or serum.

DISCUSSION

There is little doubt that the great concentration of the blood observed in these experiments was due to the filtration of fluid from the blood into the tissues of the dependent parts, as evidenced by the increase in leg volume. In our experiments, however, none of the strictly normal subjects developed pitting edema in spite of filtering pressures which should, unopposed, cause enough fluid to be filtered in the time the subjects stood to induce such an edema. Landis and Gibbon (8) have shown that filtration of fluid from the vessels (arm) begins at a venous pressure of about 15 cm. of water and increases at the rate of around 0.0033 cc. per minute per 100 cc. of tissue (arm) for every 1 cm. rise in pressure In our normal subjects venous pressures in the foot of 96.6 to 120 cm. of water were recorded. Undoubtedly, as Krogh and his associates (9) have shown, the rising colloid osmotic pressure, itself due to the loss of fluid from the blood, automatically diminishes filtration. This is, however, insufficient to stop filtration short of edema and will oppose only a part of the effective filtering pressure. Other mechanisms must operate to limit filtration, the most important of which appears to be an increasing tissue pressure which rises with the accumulation of extravascular fluid. Attempts at direct measurement of tissue pressure proved unsatisfactory but Landis and Gibbon (8) have found that the accumulation of 1 cc. of fluid per 100 cc. of tissue (arm) will raise the tissue pressure sufficiently to slow filtration .033 cc. per minute per 100 cc. of tissue, corresponding to a reduction in venous

pressure of 10 cm. of water. Five cc. of extravascular fluid per 100 cc. of tissue slows filtration 1.10 cc. per minute and is equal to a reduction of 35 cm. of water in venous pressure. On the basis of these results we have calculated the assumed tissue pressures at the end of the standing period (Table IV). The method is as follows: The calculations of the tissue pressure and of the effective filtering and antifiltering pressures are based on the observations of Krogh, Landis and Turner (9) and Landis and Gibbon (8). It has been necessary to assume (1) that these authors' constants of filtration and retardation of filtration for the arm are applicable to the leg of the standing individual; (2) that the colloid osmotic pressure of venous blood from the foot is, on the average, the same as that of capillary blood of the leg; that the increase of mean capillary pressure of the leg is two-thirds the venous pressure as measured at the level of the foot and represents the average hydrostatic pressure of that part of the leg whose volume was measured. The level representing the average venous pressure in the part of the leg whose volume was measured was taken to be the mid-point of the boot. This was roughly one-third the distance from the point at which venous pressure was measured to the xiphoid; (3) that the value of the mean venous pressure above which filtration of fluid occurs is $3/7 \, \pi_1$ when π_1 is the initial colloid osmotic pressure and π_2 the final colloid osmotic pressure. (The average normal colloid osmotic pressure while reclining is taken to be 35.0 cm. Filtration begins at a venous pressure of approximately 15 cm. of H_2O (Krogh, Landis and Turner) which is 3/7 of the osmotic pressure); (4) that the increase in colloid osmotic pressure plus the other changes (tissue pressure) have produced a balance at the end of the observation so that equilibrium exists. Then, where V is the venous pressure at the foot, $(2/3 \, V - 3/7 \, \pi_1)$ should be equal to $(\pi_2 - \pi_1) +$ increase in tissue pressure. The increase in tissue pressure is approximately 10 cc. of water for each 1 cc. of fluid per 100 cc. of leg. Hence $2/3$ $V - 3/7 \, \pi_1$ (filtering pressure) should equal $\pi_2 - \pi_1 + 10 \, F$ (antifiltering pressure) where F equals in cc. the increased fluid per 100 cc. of leg at the end of the experiment. If the above is not approximately true then (1) equilibrium has not been established, (2) some other factor must come in to balance the equation, (3) any or all the above assumptions are wrong. Attention is directed to the large part which tissue pressure apparently plays in limiting the escape of fluid from the vessels. In most of the subjects this pressure was at least twice as great as the effective colloid osmotic pressure. In Subject 11, with nephrosis and a severe grade of edema, it was twenty-seven times greater and in the " normal " subject who developed pitting edema (Subject 5) more than four times greater.

If the rising colloid osmotic pressure of the blood and the increasing tissue pressure are the primary factors restricting filtration, then, at the point of equilibrium, their combined pressures should equal or slightly exceed the filtering pressure. In Table IV, we have compared the effective

TABLE IV

Comparison of the effective filtering pressure and the combined effective osmotic and tissue (antifiltering) pressures at the end of the experiment *

Subject	Effective osmotic pressure	Effective tissue pressure	Combined effective osmotic and tissue (antifiltering) pressures	Filtering pressure	Standing time
Normals	*cc. water*	*cc. water*	*cc. water*	*cc. water*	*minutes*
1	14.8	42.8	57.6	45.8	62
2	22.4	46.3	68.7	58.0	63
3†	15.8	48.7	64.5	55.0	43
4	11.0	31.1	42.1	49.7	66
5	8.6	36.1	44.7	67.0	90
Patients					
7	8.3	42.9	51.2	53.2	75
8	14.9	30.7	45.6	52.7	110
11‡	1.4	37.9	39.3	37.5	85

* The method of calculating these pressures is given in the body of the paper.
† Could not stand continuously.
‡ Sat in chair.

filtering pressure with the antifiltering pressure (combined effective colloid osmotic and tissue pressure) at the end of the experiment in those experiments in which the data are available. In three of the five normal subjects the forces opposed to filtration were, at the end of the experimental period, larger than the filtering pressure by a greater or less amount. In two the filtering pressure was the greater and it is probable that filtration had not ceased. Whether in Subject 4, further standing would have resulted in an equilibrium with the production of a pitting edema cannot be determined. From simple inspection and palpation she seemed close to such a state. Subject 5 did show a definite though slight pitting edema at this stage even though equilibrium had apparently not been reached. One of the patients showed an excess of pressure opposed to filtration suggesting that filtration had been stopped at the level of pitting edema existing at the end of the experiment. In the others filtration may still have been occurring.

It may be doubted whether these calculations are justified or the results significant in view of the relatively inaccurate method of measuring leg volume and the number of assumptions which were necessary. Nevertheless, we believe that the magnitude of the changes are sufficient to allow for a considerable error without seriously impairing the significance of the results. Even though there may be large errors in the tissue pressures and in the filtering pressures as we have calculated them, the magnitude of the colloid osmotic pressures, which are much more accurately determined, indicates that some other factor, presumably tissue pressure, was of much

greater importance in limiting filtration than was the increase in colloid osmotic pressure.

According to Thompson et al. (10), equilibrium in the standing position is reached after about 30 minutes. While this may in general be true our results suggest that individual variations occur and that individuals who have lower serum colloid osmotic pressures or whose tissues are relaxed and less well filled with extravascular fluid may require a longer time to reach equilibrium. In persons with serum colloid osmotic pressures close to, or slightly below, the lower normal limits equilibrium might not be reached until sufficient fluid had accumulated in the tissues to cause pitting edema. For example, Subject 5, with a serum protein concentration and colloid osmotic pressure just below normal values, did actually develop slight pitting edema and had apparently not reached equilibrium after 90 minutes. On the other hand individuals with more than the usual amount of fluid in the tissues or with unusually high osmotic pressures might establish equilibrium in a shorter time than 30 minutes. It should be emphasized that equilibrium as referred to here is to be understood as existing for a limited time only. With continued high filtering pressures (standing) it is probable that the tissues would stretch, permitting further filtration.

It will be observed (Table I) that in the normal subjects the percentage increases in the concentration of total protein, albumin and globulin respectively were very nearly the same. This might be taken as evidence that the vessels in those subjects were impermeable to plasma proteins on the assumption that had they been permeable they would have been more permeable to the smaller albumin molecule. This should have resulted in a relatively greater concentration of globulin than albumin had there occurred any significant leak of proteins through the capillary wall. The assumption that the capillaries are practically impermeable to the proteins under conditions similar to those in our experiments has been made by Thompson, Thompson and Dailey (10) and by Krogh, Landis and Turner (9). Waterfield (11) found, however, that standing caused not only a loss of fluid from the blood but considerable amounts of protein as well, while Drinker and his associates (12) believed that the capillaries are quite permeable to the proteins even at venous pressures much lower than obtained in our experiments. More recently Landis and his associates (7), using the arm and artificially raised venous pressures, have shown that while the capillaries under normal conditions are nearly impermeable to protein up to venous pressures of 60 mm. of mercury (71.6 cm. H_2O), above that pressure significant losses occur. At venous pressures of 80 mm. of mercury losses as high as 2.8 grams per 100 cc. of filtrate were found. No difference in permeability of the protein fractions as shown by consistent differences in their percentage increase in concentration in the blood was observed in their experiments or in those of Plass and Rourke

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fluid was removed under the circumstances. The influence of muscular contractions was, of course, inoperative.

The unexpected finding that patients with a relatively constant amount of chronic edema behaved essentially as normal subjects in spite of lower osmotic pressures and an excess of extravascular fluid may be explained by an adjustment to the edema (stretching) which resulted in tissue pressures similar to the normal. Under these conditions the changes in concentration of the blood with changes in posture would be relatively the same unless the amount of edema was rapidly changing, either increasing or decreasing.

In spite of high filtering pressures it is probable that standing for approximately one hour will not cause pitting edema in the legs or feet of most normal persons. However, persons with slightly lowered serum proteins, as Subject 5, might well develop edema under these conditions. Although the experimental conditions are severe, if the factor of time is considered our results suggest that normal ambulatory activity might produce pitting edema in the legs of such individuals. In this case the loss of fluid from the blood might, at certain times, raise an initial serum protein concentration from slightly below normal to within normal limits.

SUMMARY

Standing at an angle of 75 degrees for approximately one hour caused an increase of 18 to 40 per cent in the concentration of serum protein and an increase of 29 to 65 per cent in the colloid osmotic pressure of the blood in the foot of normal subjects. The percentile increase in the total protein concentration and that of the two protein fractions were practically identical. The increase in the concentration of protein and in the osmotic pressure was approximately twice as great as occurred in the blood in the arm. The volume of the leg increased from 3.11 to 4.87 cc. per 100 cc. of leg but pitting edema did not occur in any of the strictly normal subjects.

Patients with chronic (nutritional) edema showed essentially similar changes with a demonstrable increase in the pitting edema. Calculations based on the data obtained suggest that an increase in tissue pressure was three to five times as important in limiting the loss of fluid from the blood as was the increase in colloid osmotic pressure.

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EPINEPHRINE HYPERGLYCEMIA

WITH PARTICULAR REFERENCE TO THE ARTERIOVENOUS
BLOOD SUGAR DIFFERENCE IN HEPATIC DISEASE

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Interest in the significance of the post-absorptive arteriovenous blood sugar difference was stimulated by the early observations of Hagedorn (1), Holst (2), Foster (3) and Henriques and Ege (4). The great advances made during the past decade in our knowledge of carbohydrate metabolism have thrown considerable light upon the mechanisms involved in the production of normal and abnormal blood sugar responses to the ingestion of glucose. Friedenson, Rosenbaum, Thalheimer and Peters (5) believe that the initial rise of arterial and venous blood sugar is an expression of the absorption of glucose from the intestine. The subsequent fall in the bood sugar concentration is the result of two factors: (1) removal of sugar by the liver to form glycogen and (2) removal of sugar by the tissues, especially muscle, for the formation of glycogen and for combustion. The arteriovenous blood sugar difference, i.e., the difference between the quantity of sugar supplied to and that leaving the tissues, is naturally assumed to represent the quantity stored or otherwise utilized in the tissues. These authors state that one should expect certain abnormalities in the curve of alimentary glycemia in individuals with hepatic disease: namely, an excessively high or prolonged blood sugar response, because glucose is not removed in the normal manner from the blood by the liver, but with retention of the normal arteriovenous difference, the ability of the tissues to utilize glucose being unimpaired. The observations of Friedenson and his associates (5) were well in accord with these theoretical expectations. However, it was found that the range of normal variation was so wide and the deviation from the normal in some cases of hepatic disease so slight, that this method of investigation was considered to be of doubtful value in the study of such patients.

The effect of epinephrine on carbohydrate metabolism has been rather clearly demonstrated in recent years although certain important phases of its action are still obscure. The extensive literature which has arisen in this connection cannot be reviewed here in detail; an excellent discussion of the subject is available in the recent review by Cori (6). A few sig-

nificant points may, however, be mentioned. In 1906, Velich (7) found that epinephrine glycosuria, first demonstrated by Blum in 1901 (32), did not develop in the hepatectomized frog. The important part played by the liver in the production of epinephrine hyperglycemia was also demonstrated by Mann (8) and Soskin (9), who showed that the injection of epinephrine has no effect on the blood sugar of hepatectomized dogs. Observations such as these naturally suggest that the rise in blood sugar caused by this substance is due to increased glycogenolysis in the liver.

It soon became apparent, however, that this factor alone does not suffice to explain the effects of epinephrine on carbohydrate metabolism (viz. the observation of Cori and Cori (10a) that the liver glycogen content of rats to which glucose was administered remained unchanged or was increased after the injection of epinephrine). Bodo, Benaglia and Friedman (11) found that a post-epinephrine increase in hepatic glycogen occurred only in animals (dogs) fasted for 6 to 14 days, a definite decrease being noted in fed animals. The preponderance of evidence, however, is in accord with the findings of Cori and Cori (10a). The work of Cori and Cori (10b, c), Zimmermann (12), Geiger (13), Corkill and Marks (14), Goldblatt (15) and others indicates rather definitely that acceleration of glycogenolysis in the muscles, with decrease in their glycogen content, is one of the significant physiological effects of epinephrine. Cori (6) argues that the fundamental action of this hormone in liver and muscle is the same, namely, increased glycogenolysis, the chief end product in the liver being glucose and in the muscles lactic acid. The decrease in muscle glycogen after the administration of epinephrine is due to the fact that glycogenolysis proceeds more rapidly than new formation of muscle glycogen; the secondary increase in liver glycogen is due to the fact that, after a brief interval, hepatic glycogen formation proceeds more rapidly than hepatic glycogenolysis. The action of epinephrine may be therefore summarized as follows: increased glycogenolysis in the liver and muscles, decreased carbohydrate utilization in the muscles, with consequent increased formation of lactic acid which subsequently increases the glycogen content of the liver. Loeb, Reeves and Glasier (16) state that epinephrine hyperglycemia results from an initial, transient glycogenolysis in the liver, the hyperglycemia being then maintained by decreased glucose utilization in the muscles.

Markowitz (17) and Olmsted and Coulthard (18) concluded that the blood sugar response to the injection of epinephrine depends to a considerable degree upon the glycogen content of the liver. Brill (19), Brill and Fitz-Hugh (20), Kugelmann (21) and Loeb, Reeves and Glasier (16) found that this response was not as great in individuals with hepatic disease as in normal persons, who show an average increase of 35 to 45 mgm. per 100 cc., usually within one-half to one hour. However, there was no apparent definite correlation between the severity of liver damage and the

blood sugar response, and the findings in border-line cases with slight hepatic lesions were in many cases essentially normal.

We have studied the concentration of sugar in capillary and venous blood following the injection of epinephrine in 16 individuals without and 31 patients with some lesion of the liver or bile passages. Blood sugar determinations were made upon capillary and venous blood obtained simultaneously in the fasting state and 30, 60 and 120 minutes after the intramuscular injection of 1 cc. of a 1–1000 solution of epinephrine, the site of injection being thoroughly massaged. The 1931 micro method of Benedict (22) was employed in all determinations, being checked by parallel determinations by the 1931 macro method of Benedict (22) upon the venous blood samples. In accordance with the findings of the great majority of workers, the sugar content of capillary blood, obtained by deep puncture of the finger, is regarded as practically identical with that of arterial blood and is designated " arterial-blood " sugar for purposes of convenience. Jonas (23) has recently reported a considerable variation between arterial and capillary blood sugar values but this is contrary to the experience of the majority of workers, particularly if care is exercised to insure a deep puncture.

The experimental material was as follows: (1) 16 patients with no evidence of disease of the liver or bile passages; (2) 6 patients with noncalculous cholecystitis; (3) 5 patients with cholelithiasis; (4) 2 patients with obstructive jaundice due to carcinoma of the pancreas; (5) 4 patients with advanced carcinoma of the liver, one primary and three secondary; (6) 6 patients with " catarrhal " jaundice; (7) 2 patients with postarsphenamine jaundice; (8) 6 patients with portal cirrhosis. The detailed findings are presented in the accompanying charts.

No hepatic or biliary tract disease (16 cases)

The data obtained in this group are presented in Table I. The fasting arterial blood sugar concentration varied between 63 and 95 mgm. (average 81) and the venous blood sugar between 62 and 93 mgm. (average 79) per 100 cc. In our experience, the normal range by the method employed is from 60 to 105 mgm. per 100 cc. Following the administration of epinephrine, the maximum rise above the resting level ranged from 34 to 74 mgm. (average 47) in the arterial blood and 28 to 63 mgm. (average 36) in the venous blood. The maximum increase in the arteriovenous blood sugar difference, above that present in the fasting state, ranged from 3 to

29 mgm. (average 14) per 100 cc. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 6 cases, and in the 30 minute sample in 10 cases. The maximum arteriovenous blood sugar difference was present at 60 minutes in 10 cases, at 30 minutes in 4 cases and at 120 minutes in 2 cases.

TABLE I

Extra-hepatic disease

Case	Condition	Serum bilirubin*	Brom-sulph-alein retention	Blood sugar						
					Minutes after epinephrine				Maximum rise	Maximum increase A-V†
					0	30	60	120		
		mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
C. E.	Hysteria	0.36	0	A*	86	121	133	102	47	16
				V*	86	110	117	88	31	
L. O.	Normal			A	90	160	151	116	70	9
				V	90	152	142	107	62	
A. B.	Emphysema	0.48	0	A	82	117	133	83	51	21
				V	82	100	112	86	30	
A. P.	Convalescent pneumonia			A	63	137	133	76	74	24
				V	62	125	108	66	63	
L. M.	Osteoarthritis			A	80	142	133	102	62	16
				V	80	133	117	100	53	
W. F.	Autonomic imbalance			A	83	117	105	90	34	3
				V	76	111	100	80	35	
S. L.	Sacro-iliac sprain	0.36	0	A	72	101	136	101	64	16
				V	69	82	119	85	50	
E. B.	Ureteral calculus	0.26	0	A	84	106	148	104	64	19
				V	81	95	126	88	45	
A. R.	Psychoneurosis			A	74	131	114	68	57	14
				V	74	117	100	62	43	

TABLE I (*continued*)

Case	Condition	Serum bilirubin*	Bromsulphalein retention	Blood sugar						
					Minutes after epinephrine				Maximum rise	Maximum increase A-V†
					0	30	60	120		
		mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
R. C.	Prostatitis	0.51	0	A	75	127	112	80	52	18
				V	74	119	93	74	45	
G. W.	Osteoarthritis			A	80	117	111	100	37	11
				V	80	108	100	95	28	
P. S.	Emphysema	0.44	0	A	95	125	142	125	47	12
				V	90	111	133	108	43	
H. H.	Neurosis	0.43	0	A	73	140	108	86	67	21
				V	71	117	86	66	46	
M. B.	Colitis	0.3	0	A	95	151	133	107	56	18
				V	93	131	125	95	38	
V. M.	Anthracosis			A	78	111	142	90	64	29
				V	77	100	112	76	35	
W. B.	Mitral stenosis	0.25	0	A	83	131	111	91	50	8
				V	82	125	102	83	43	
Average				A	81	127	128	95	47	14
				V	79	115	112	85	36	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

Non-calculous cholecystitis (6 cases)

The data obtained in this group are presented in Table II. The fasting arterial blood sugar concentration varied between 73 and 111 mgm. (average 92) and the venous blood sugar between 71 and 110 mgm. (average 92) per 100 cc. Following the administration of epinephrine, the maximum rise above the resting level ranged from 17 to 70 mgm. (average 33) in the arterial blood and from 12 to 56 mgm. (average 28) in the venous

blood. The maximum increase in the arteriovenous blood sugar difference, above that present in the fasting state, ranged from 3 to 27 mgm. (average 10) per 100 cc. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 3 cases, in the 30 minute sample

TABLE II

Cholecystitis

Case	Serum bilirubin	Bromsulphalein retention	Blood sugar							
				Minutes after epinephrine				Maximum rise	Maximum increase A-V†
				0	30	60	120		
	mgm. per 100 cc.	*per cent*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
W. S.	0.34	0	A*	93	121	149	104	56	14
			V*	95	133	137	100	42	
D. S.	0.6	0	A	90	129	105	76	39	4
			V	90	125	102	72	35	
J. R.	1.32	0	A	111	181	142	115	70	14
			V	110	166	132	111	56	
M. C.	0.55	0	A	73	80	90	80	17	8
			V	71	83	80	74	12	
F. L.	0.52	0	A	92	131	139	99	47	27
			V	93	105	116	90	23	
J. K.	2.68	55	A	95	111	121	104	26	3
			V	93	111	119	99	26	
Average			A	92	125	124	96	33	10
			V	92	120	114	91	28	

* A = arterial blood; V = venous blood.

† Maximum increase in arteriovenous difference.

in 2 cases and in 1 case the maximum arterial blood sugar value was attained at 60 minutes and the maximum venous blood sugar value at 30 minutes. The maximum arteriovenous difference was present at 60 minutes in 2 cases, at 30 minutes in 3 cases and at 120 minutes in 1 case.

Cholelithiasis (5 cases)

The data obtained in this group are presented in Table III. The fasting arterial blood sugar concentration varied between 82 and 106 mgm. (average 90) and the venous blood sugar between 82 and 105 mgm. (aver-

TABLE III

Cholelithiasis

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar					
				Minutes after epinephrine				Maximum rise	Maximum increase A-V†
				0	30	60	120		
	mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
L. P.	1.45	60	A*	82	90	104	86	22	8
			V*	82	89	100	78	18	
H. C.	2.2	35	A	88	114	130	97	42	12
			V	86	109	123	83	37	
J. N.	5.6	100	A	106	143	163	129	57	11
			V	105	131	153	118	48	
A. C.	1.28	15	A	85	112	133	111	48	13
			V	85	99	121	100	36	
T. F.	41.6	100	A	87	160	147	94	60	6
			V	86	153	144	93	58	
Average			A	90	124	135	103	45	8
			V	89	116	128	94	39	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

age 89) per 100 cc. Following the administration of epinephrine, the maximum rise above the resting level ranged from 22 to 60 mgm. (average 45) in the arterial blood and from 18 to 58 mgm. (average 39) in the venous blood. The maximum increase in the arteriovenous blood sugar difference, above that present in the fasting state, ranged from 6 to 13 mgm. (average 8) per 100 cc. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 4 cases and in the 30 minute

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sample in 1 case.   The maximum arteriovenous difference was present at 30 minutes in 3 cases and at 120 minutes in 2 cases.

Carcinoma of pancreas (2 cases)

The data obtained in this group are presented in Table IV.   The fasting arterial blood sugar concentrations were 108 and 112 mgm. (average 110) and the venous blood sugars 111 and 114 mgm. (average 112) per

TABLE IV

Carcinoma of pancreas

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar					
				Minutes after epinephrine ·				Maxi-mum rise	Maxi-mum in-crease A-V†
				0	30	60	120		
	mgm. per 100 cc.	*per cent*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
A. R.	27.2	100	A*	108	123	137	115	29	8
			V*	111	118	133	114	22	
J. S.	16.7	100	A	112	147	166	148	54	16
			V	114	133	155	141	41	
Average			A	110	135	151	131	41	12
			V	112	125	144	127	32	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

100 cc.   Following the administration of epinephrine, the maximum rise above the resting level was 29 and 54 mgm. (average 41) in the arterial blood and 22 and 41 mgm. (average 32) in the venous blood.   The maximum increase in the arteriovenous difference, above that present in the fasting state, was 8 and 16 mgm. (average 12) per 100 cc., respectively. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in both cases.   The maximum arteriovenous difference was present at 30 minutes in both cases.

Carcinoma of liver (4 cases)

The data obtained in this group are presented in Table V.   The fasting arterial blood sugar concentration varied between 63 and 111 mgm. (average 81) and the venous blood sugar between 62 and 111 mgm. (average

TABLE V

Carcinoma of liver

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar					
				Minutes after epinephrine				Maximum rise	Maximum increase A-V†
				0	30	60	120		
	mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
D. B.	1.72	55	A*	63	64	60	59	1	0
			V*	62	64	59	58	2	
J. C.	8.0	40	A	71	82	88	87	17	7
			V	68	72	83	79	15	
C. L.	4.0	80	A	80	90	95	86	15	0
			V	71	83	86	81	15	
F. M.	1.92	30	A	111	117	129	114	18	4
			V	111	113	125	111	14	
Average			A	81	88	93	86	12	2
			V	78	83	88	82	10	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

78) per 100 cc. Following the administration of epinephrine, the maximum rise above the resting level ranged from 1 to 18 mgm. (average 12) in the arterial blood and from 2 to 15 mgm. (average 10) in the venous blood. The maximum increase in the arteriovenous difference, above that present in the fasting state, ranged from 0 to 7 mgm. (average 2) per 100 cc. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 3 cases and in the 30 minute sample in 1 case. The maximum arteriovenous difference was present at 30 minutes in the 2 cases in which an increase in this factor was noted.

"Catarrhal jaundice" (6 cases)

The data obtained in this group are presented in Table VI. The fasting arterial blood sugar concentration varied between 86 and 99 mgm. (average 93) and the venous blood sugar between 84 and 99 mgm. (average 91) per 100 cc. Following the administration of epinephrine, the

TABLE VI

Catarrhal jaundice

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar					
				Minutes after epinephrine				Maximum rise	Maximum increase A-V†
				0	30	60	120		
	mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
R. H.	13.32	100	A*	93	136	112	100	43	5
			V*	93	133	111	95	40	
C. T.	11.0	100	A	95	108	106	100	13	13
			V	94	102	105	86	11	
M. K.	14.1	100	A	86	100	136	104	50	5
			V	84	101	132	97	48	
E. H.	18.6	100	A	92	95	102	103	11	4
			V	88	91	96	95	8	
F. H.	15.33	100	A	99	105	121	95	22	9
			V	99	105	114	86	15	
M. D.	3.52	10	A	91	111	119	97	28	3
			V	89	106	114	92	25	
Average			A	93	109	116	100	23	6
			V	91	106	112	92	21	

* A = arterial blood; V = venous blood.

† Maximum increase in arteriovenous difference.

maximum rise above the resting level ranged from 11 to 50 mgm. (average 23) in the arterial blood and from 8 to 48 mgm. (average 21) in venous blood. The maximum increase in the arteriovenous difference, above that present in the fasting state, ranged from 3 to 13 mgm. (average 6) per 100 cc. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 3 cases, in the 30 minute sample in 1 case; in 2 instances the maximum arterial values occurred at 30 and 120 minutes respectively and the maximum venous values at 60 minutes. The maximum arteriovenous difference was present at 30 minutes in 1 case and at 120 minutes in 5 cases.

Post-arsphenamine jaundice (2 cases)

The data obtained in this group are presented in Table VII. The fasting arterial blood sugar concentrations were 71 and 72 mgm. and the venous blood sugars 62 and 70 mgm. per 100 cc. Following the adminis-

TABLE VII

Post-arsphenamine jaundice

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar						
				Minutes after epinephrine				Maxi-mum rise	Maxi-mum in-crease A-V†
				0	30	60	120		
	mgm. per 100 cc.	per cent		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
R. B.	12.8	100	A*	71	74	80	66	9	0
			V*	62	71	73	64	11	
J. B.	10.4	100	A	72	78	86	75	14	4
			V	70	76	80	69	10	
Average			A	71	76	83	70	12	2
			V	66	73	76	66	10	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

tration of epinephrine, the maximum rise above the resting level was 9 and 14 mgm. in the arterial blood and 11 and 10 mgm. in the venous blood. The maximum increase in the arteriovenous difference, above that present in the fasting state, was 0 and 4 mgm. per 100 cc. respectively. The peak of both arterial and venous blood sugar curves occurred in the 60 minute sugar sample in both cases.

Portal cirrhosis (6 cases)

The data obtained in this group are presented in Table VIII. The fasting arterial blood sugar concentration varied between 78 and 92 mgm. (average 85) and the venous blood sugar between 74 and 86 mgm. (average 81) per 100 cc. Following the administration of epinephrine, the maximum rise above the resting level ranged from 10 to 24 mgm. (average 16) in the arterial blood and from 9 to 25 mgm. (average 15) in the venous blood. The maximum increase in the arteriovenous difference, above that present in the fasting state, ranged from 0 to 15 mgm. per 100 cc. The

TABLE VIII

Portal cirrhosis

Case	Serum bilirubin	Brom-sulph-alein retention		Blood sugar					
				Minutes after epinephrine				Maximum rise	Maximum increase A-V†
				0	30	60	120		
	mgm. per 100 cc.	*per cent*		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
J. F.	2.6	15	A*	90	93	100	96	10	0
			V*	83	93	97	97	14	
A. B.	1.2	40	A	82	87	90	95	13	4
			V	82	85	89	91	9	
F. W.	0.56	0	A	78	102	93	86	24	15
			V	78	100	82	71	22	
S. K.	1.6	20	A	78	88	98	88	20	4
			V	74	80	94	84	20	
H. M.	0.44	0	A	90	97	114	106	24	2
			V	86	95	111	100	24	
G. R.	2.43	55	A	90	102	111	86	21	7
			V	85	90	104	84	19	
Average			A	85	95	101	93	16	1
			V	81	90	96	88	15	

* A = arterial blood; V = venous blood.
† Maximum increase in arteriovenous difference.

peak of both arterial and venous blood sugar curves occurred in the 60 minute sample in 4 cases, in the 30 minute sample in 1 case and in the 120 minute sample in 1 case. The maximum arteriovenous difference was present at 30 minutes in 2 cases and at 120 minutes in 3 cases.

DISCUSSION

Our findings with regard to the changes in the arteriovenous blood sugar difference in individuals without hepatic disease differ from those of Wiechmann (24), Cori and Cori (25) and Samson and Jacobs (26).

These authors observed no increase in the arteriovenous difference in normal men and animals following the injection of epinephrine and concluded that the increased tissue utilization of glucose, which occurs during alimentary hyperglycemia, does not take place during epinephrine hyperglycemia. We cannot explain this discrepancy. However, Jonas (23), in a few instances, noted an increase above the fasting difference one hour after the injection of 1 cc. of epinephrine. Furthermore, the observations of Samson and Jacobs (26) were made at intervals of several hours after the administration of epinephrine was begun and at subsequent varying intervals during its continuous injection into the blood stream. Their data are therefore not comparable to those reported here, which represent a rapid and transient response to the injection of this agent. In connection with the observed variations in the hepatic glycogen content following epinephrine administration, Cori (6) has pointed out that data of this sort can be compared only if obtained under identical conditions, and that the basic action of a hormone in one direction is often obscured by simultaneous or subsequent changes which it produces in other directions.

Our findings with regard to the degree of epinephrine hyperglycemia in individuals without hepatic disease are in accord with those of other observers previously referred to. The glycemic response in the patients with cholecystitis, cholelithiasis and carcinoma of the pancreas was extremely variable and was apparently unrelated to the degree of hyperbilirubinemia or bromsulphalein retention. If the extent of the initial hyperglycemia is assumed to be an index of the readily available glycogen reserve of the liver, this lack of correlation may be regarded as indicative of dissociation of impairment of excretory and metabolic functions of the liver in disease of the biliary tract. This has been emphasized by Cantarow (27), Althausen (28) and Cantarow and Gehret (29). A few of the cases of cholecystitis (Table II) and cholelithiasis (Table III) and one case of carcinoma of the pancreas (Table IV) showed a venous blood sugar response which might be regarded as subnormal (increase less than 30 mgm. per 100 cc.). A subnormal response was observed in similar cases by Loeb, Reeves and Glasier (16).

Although there was a marked degree of variation in the maximum increase in the arteriovenous blood sugar difference, the average values in the groups of patients with cholecystitis and cholelithiasis were slightly below that observed in the non-hepatic group. However, no significance can be attached to this fact because of the small number of cases and the extreme variation in the findings. One point of interest, however, is the fact that the increase in the arteriovenous difference bore no apparent relation to the rise in either the arterial or the venous blood sugar level. This was also true of the control group. It may be of interest to note that Case W. F. of the latter group, in which the arteriovenous difference increased only 3 mgm. per 100 cc., had marked autonomic imbalance, which may con-

ceivably have influenced the response to epinephrine. Excluding this case, the smallest increase in the A–V difference in the control group was 8 mgm. per 100 cc.

Of the group of 18 patients with various forms of intrahepatic disease, including metastatic and primary carcinoma of the liver (Table V), "catarrhal jaundice" (Table VI), post-arsphenamine jaundice (Table VII), and portal cirrhosis (Table VIII), in only 2 did the venous blood sugar increase 30 mgm. or more per 100 cc.; both of these were patients with "catarrhal jaundice" (Cases R. H. and M. K., Table VI). In the remainder, the blood sugar curves were essentially the same as those obtained by Brill and Fitz-Hugh (20), Brill (19), Kugelmann (21), and Loeb, Reeves and Glasier (16) in individuals with advanced intrahepatic disease. In these cases, too, the discrepancies between the degree of epinephrine hyperglycemia, bilirubinemia and retention of bromsulphalein are striking. Of particular interest in these groups of patients are the changes in the arteriovenous blood sugar difference. The maximum increase in this fraction was below 8 mgm. per 100 cc. in all but 3 cases; two of these were patients with "catarrhal jaundice" (Cases C. T. and F. H., Table VI) and one was a patient with portal cirrhosis (Case F. W., Table VIII). Essentially normal findings were obtained in 3 of the 6 patients with "catarrhal jaundice" upon repetition of the studies following recovery. The average values for this group during the height of the attack and during convalescence are presented in Figures 1 and 2.

FIG. 1. AVERAGE POST-EPINEPHRINE ARTERIAL (A) AND VENOUS (V) BLOOD SUGAR VALUES IN 6 PATIENTS WITH "CATARRHAL JAUNDICE"

The significance of these observations is conjectural. They confirm the findings of previous investigators with regard to the subnormal glycemic response to epinephrine in patients with intrahepatic disease. We believe that the subnormal response in occasional patients with cholecystitis and cholelithiasis may be of significance in suggesting possible depletion of hepatic glycogen, even though no evidence of hepatic disease may be

demonstrable clinically.   It is of interest to note that although Loeb and his associates ، (16) obtained in patients with hepatic disease results like those observed in other patients with a variety of disorders, these conditions were in many instances of such a nature as might be expected to be associated with a low hepatic glycogen content.   These authors advance the hypothesis that this subnormal response to epinephrine may be dependent, not upon depleted hepatic glycogen stores but upon inhibition of certain epinephrine effects by factors still unknown.   Although this view is sup-

FIG. 2. AVERAGE POST-EPINEPHRINE ARTERIAL (*A*) AND VENOUS (*V*) BLOOD SUGAR VALUES IN 3 PATIENTS CONVALESCING FROM "CATARRHAL JAUNDICE"

ported by their studies of blood lactic acid in these conditions, the former and more widely accepted opinion is the more plausible.

The frequently subnormal A–V difference in patients with intrahepatic disease is incapable of satisfactory explanation at the present time.   A very logical explanation would be that the extent of removal of glucose from arterial blood in the tissues is diminished in such cases because the degree of elevation of the arterial blood sugar concentration is subnormal.   Holst (2) stated that the magnitude of the A–V difference varies directly with the existing degree of hyperglycemia following the ingestion of glucose. However, this view is not supported by the observations of Friedenson and his associates (5) nor by the data presented here.   This factor may, however, play some part in the production of this deviation from the normal response.   The decreased A–V difference is suggestive of inhibition of glucose utilization by the muscles, which is generally recognized as one of the

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important effects of epinephrine upon carbohydrate metabolism. Why this particular action should be exaggerated in the presence of hepatic disease, however, is difficult to understand. The point raised by Soskin, Priest and Schutz (30) may be of importance in this connection. These observers state that, according to the dosage employed, epinephrine either increases or decreases the blood flow through the muscles; this variability, they say, together with the hemoconcentrating effect of the hormone, renders inaccurate any observations of the arteriovenous blood sugar difference. Although the dosage of epinephrine employed in this study was identical in all cases, the well-recognized individual variation in the vascular response to this hormone, particularly in disease states, may have been responsible in part for the supposed changes in the A–V blood sugar difference in patients with intrahepatic disease. On the other hand, it may be that these changes are dependent upon decreased peripheral utilization of glucose resulting from diminished insulin activity. This may be due to a quantitative decrease in what Himsworth (31) has termed insulin-kinase, the hypothetical "activator" of insulin, which he believes to be diminished in the presence of intrahepatic disease.

SUMMARY

1. Studies were made of the deep capillary (arterial) and venous blood sugar concentration before and 30, 60 and 120 minutes after the injection of 1 cc. of epinephrine in 16 individuals without and 31 patients with some lesion of the liver or bile passages.

2. In the control group, the arterial blood sugar showed an average increase of 47 mgm. and the venous blood sugar 36 mgm. per 100 cc. The arteriovenous blood sugar difference showed an average maximum increase of 14 mgm. per 100 cc. above the resting level.

3. The glycemic response in patients with cholecystitis, cholelithiasis and pancreatic carcinoma was extremely variable and was apparently unrelated to the degree of hyperbilirubinemia or bromsulphalein retention. A subnormal response was obtained in 5 cases in these groups.

4. Of the group of 18 patients with various forms of intrahepatic disease, including metastatic and primary carcinoma of the liver, "catarrhal jaundice," portal cirrhosis and post-arsphenamine jaundice, only 2 exhibited a normal glycemic response to epinephrine. There was a striking discrepancy between the degree of epinephrine hyperglycemia and that of bilirubinemia and bromsulphalein retention.

5. The post-epinephrine increase in the arteriovenous blood sugar difference was extremely variable in the patients with disease of the bile passages but tended to be slightly below the average of the control group. This factor was rather consistently and markedly subnormal in the patients with intrahepatic disease.

6. It is believed that the subnormal glycemic response to epinephrine in patients with disease of the liver and bile passages is probably indicative of a state of hepatic glycogen depletion or unavailability. This may at times occur in the absence of clinically demonstrable evidence of hepatic disease.

7. The observed variations in the post-epinephrine arteriovenous blood sugar difference cannot be explained at the present time and may be of little significance. They may possibly be dependent upon diminished peripheral utilization of glucose in the presence of hepatic disease, due perhaps to decreased insulin activity.

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4. Of the group of 18 patients with various forms of intrahepatic disease, including metastatic and primary carcinoma of the liver, " catarrhal jaundice," portal cirrhosis and post-arsphenamine jaundice, only 2 exhibited a normal glycemic response to epinephrine. There was a striking discrepancy between the degree of epinephrine hyperglycemia and that of bilirubinemia and bromsulphalein retention.

5. The post-epinephrine increase in the arteriovenous blood sugar difference was extremely variable in the patients with disease of the bile passages but tended to be slightly below the average of the control group. This factor was rather consistently and markedly subnormal in the patients with intrahepatic disease.

6. It is believed that the subnormal glycemic response to epinephrine in patients with disease of the liver and bile passages is probably indicative of a state of hepatic glycogen depletion or unavailability. This may at times occur in the absence of clinically demonstrable evidence of hepatic disease.

7. The observed variations in the post-epinephrine arteriovenous blood sugar difference cannot be explained at the present time and may be of little significance. They may possibly be dependent upon diminished peripheral utilization of glucose in the presence of hepatic disease, due perhaps to decreased insulin activity.

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THE INCIDENCE AND BIOLOGICAL CHARACTERISTICS OF THE HEMOLYTIC BACILLUS COLI IN THE STOOLS OF HEALTHY INDIVIDUALS

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At the present time there is no prevailing unanimity of opinion as to the significance of the hemolytic Bacillus coli in diseases of the intestinal tract, nor as to the frequency of its occurrence in the stools of healthy individuals. Most investigators have examined the feces of normal persons for the presence of this organism merely as a part of a more extensive study of certain intestinal diseases and have reported, in the main, a lower incidence for the normal group than for those with intestinal disorders.

Schmidt (1), in 1909, examined the stools of 37 patients with diarrhea and those of 17 normal people and found the hemolytic Bacillus coli in 72.6 per cent of the former, in contrast with 65 per cent in the latter. After a careful study of these organisms, the author concluded that the hemolytic strains could be divided into three groups: (1) Those showing a clear zone of hemolysis around the colony on blood agar plates, (2) those showing a smaller zone of hemolysis and (3) those in which the hemolytic zone was only beneath the colony. In addition, Schmidt found that many of the hemolytic strains either completely or partially lost their hemolyzing property on standing, while passage through animals failed to bring about such an alteration, and, lastly, that the hemolytic organisms were scarcely more virulent for guinea-pigs than the nonhemolytic.

Dudgeon, Wordley and Bawtree (2), in 1921, were able to isolate the hemolytic form of the Bacillus coli from 11, or 35.4 per cent, of 31 cases diagnosed as " diarrhea or colitis," and from 5, or 13 per cent, of 39 " normal " stools. They found that the hemolytic urinary and fecal strains produced immune serums in rabbits more readily and showed a closer serological relationship, as a group, than the nonhemolytic strains. These writers quote some unpublished observations of Todd concerning 100 healthy infants under one year of age, among whom 13 showed hemolytic Bacillus coli in the stools. The same investigators concluded that while the hemolytic form of the Bacillus coli sometimes occurs in the feces of normal infants and adults, it is more frequently present in cases of " diarrhea or colitis."

Meyer and Löwenberg (3), in 1924, found the hemolytic form of the Bacillus coli in the intestinal tract of 25 per cent of the normal individuals examined, and in 58 per cent of the patients with intestinal disorders. In conformity with the findings of the writers previously mentioned, these investigators found, by means of agglutination tests, that the hemolytic strains formed a more or less homogeneous, serological group.

Tinozzi (4), in 1925, made a careful examination of the biological characteristics of a group of Bacillus coli and concluded that there was no difference in virulence between the hemolytic and the nonhemolytic strains and that the hemolytic organisms and also the more virulent strains of both types gave the highest agglutinations.

Dudgeon (5), in 1926, investigated the bacterial flora of the intestinal tract in health, as well as under abnormal conditions, such as chronic constipation, excessive purgation, and infection. In specimens from 200 individuals, the hemolytic type of the Bacillus coli was present in only 6 per cent, and these were all of the " pathological group." He concluded that when an abundant growth of hemolytic Bacillus coli is obtained from the feces, there is a general toxemia, together with symptoms relevant to the intestinal tract.

Davidson (6), in 1928, studied a number of Bacillus coli strains which he recovered from the stools of a group of patients suffering from pernicious anemia and from those of healthy controls, and found none that he considered definitely hemolytic.

This year, Niles and Torrey (7) have reported a careful examination of stools from a group of patients with disorders of the gastro-intestinal tract. They found that in 96 specimens the hemolytic Bacillus coli was present in 56, or 58.4 per cent, in contrast to 25 per cent in the specimens from 12 healthy adults. In 63 per cent of 46 specimens, in which the hemolytic type of Bacillus coli appeared, it was present in large numbers. Of 56 hemolytic strains, 53.6 per cent, when injected intraperitoneally into white mice, were of a virulent type, while of the 46 nonhemolytic strains examined only 23.9 per cent were found to be virulent. They, therefore, concluded that strains of the hemolytic Bacillus coli are more frequently found, and in larger numbers, in feces from patients with disorders relating to the digestive tract.

THE PRESENT STUDY

The present report is based on a study of the Bacillus coli with special reference to the hemolytic type found in the stools of a group of healthy young adults and children. The specimens supplied for this investigation were from young people, ranging in age from one month to thirty-five years, who had one or two normal bowel movements a day. All of the subjects were apparently free from disease and gastro-intestinal disturbances of any kind. Of the 73 individuals studied, 10 were children between the ages of one month and ten years, 44 were third year medical students, and the remaining 19 were doctors, technicians, or laboratory workers. In several instances, repeated stool cultures were obtained from the same person. Altogether 169 specimens were examined.

TECHNIC OF STOOL CULTURES

A small amount of the stool, about the size of a pea, was mixed with 5 cc. of sterile broth in a petri dish. A loopful of this emulsion was streaked on an Endos plate and the plate was incubated for 24 hours at 37.5° C. The Bacillus coli organisms showed up as dark red, discrete colonies with a metallic lustre on a red background. A blood agar plate was marked off, on the bottom, with a red china pencil, into one hundred small squares. By means of a small inoculating loop, 28 gauge wire, fused joint with a loop 1 mm. in diameter, a colony on the

Endos plate was lightly touched and a small streak about one-eighth of an inch long was made in the center of one of the squares. This was repeated until one hundred colonies had been subcultured. The plate was incubated at 37.5° C. for five hours, and the percentage of each type of colony present was noted. It was found that too long an incubation period at this temperature tended to cause the zones of hemolysis around the colonies to coalesce and thus the nonhemolytic colonies near them were missed. Later in the experiment, it was found that the most satisfactory results were obtained by inoculating the plates and incubating them at room temperature for about 18 hours.

The percentage of hemolytic and nonhemolytic colonies present was calculated directly from the plates (Fig. 1).
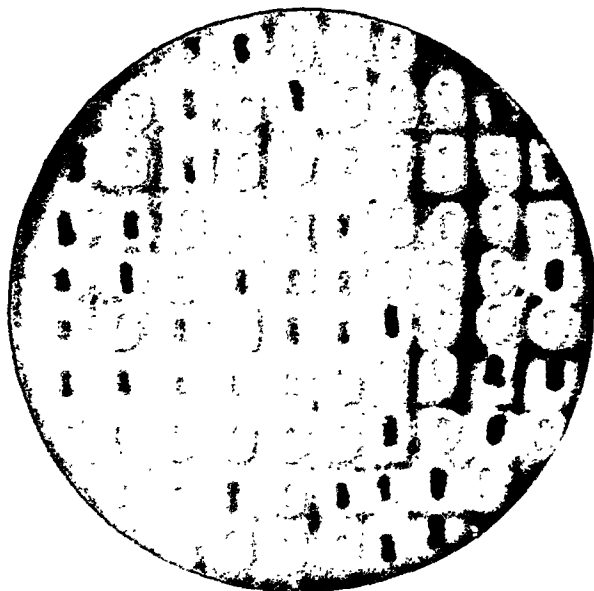
FIG. 1. PHOTOGRAPH OF A BLOOD AGAR PLATE SHOWING THE METHOD EMPLOYED FOR DETERMINING THE PERCENTAGE OF HEMOLYTIC BACILLUS COLI PRESENT IN A STOOL SPECIMEN.

RESULTS OF STOOL CULTURES

One hundred and sixty-nine stool specimens from 73 individuals, who were free from any disorders of the intestinal tract. were cultured, and the hemolytic form of Bacillus coli was present in 114, or 67.4 per cent. of the samples (Table I). In 55, or 48.2 per cent. of the 114 specimens, the hemolytic Bacillus coli were present in large numbers (50 to 100 per cent). If one considers only the first sample submitted by each individual, hemolytic Bacillus coli organisms were present in 41, or 56.2 per cent. of the 73 cases.

TABLE I

Relation of positive results for the hemolytic Bacillus coli to the number of stool specimens examined

	Number of indi- viduals	Positive for hemo- lytic Bacillus coli		Negative for hemo- lytic Bacillus coli	
		Number	Per cent	Number	Per cent
First or one sample submitted..........	73	41	56.2	32	43.8
Two or more samples submitted.......	25	22	88.0	3	12.0
Four or more samples submitted......	12	12	100.0	0	0.0

Total samples submitted were 169, of which 114, or 67.4 per cent, were positive for hemolytic Bacillus coli.

Included in the number were single fecal specimens from 10 children between the ages of one month and ten years, and hemolytic Bacillus coli were present in 8, or 80 per cent.

From 25 subjects, repeated samples were obtained, the number varying from 2 to 36. In most instances, the specimens were collected at irregular intervals, but in one case the stools were cultured daily for seven days. In 22 of the 25 individuals, hemolytic Bacillus coli were present in at least one of the specimens submitted. In each of the exceptional cases, only 2 stool cultures were examined and both were negative.

In Table II is recorded the results of the stool examinations of the 12 people who submitted 4 or more samples each. In every case except one, cultures were found that contained no hemolytic Bacillus coli, but in no instance was that type of organism absent from every sample submitted by

TABLE II

The results of repeated stool examinations on individuals submitting 4 or more specimens

Name	Total specimens	Positive for hemolytic Bacillus coli		Negative for hemolytic Bacillus coli	
		Number	Per cent	Number	Per cent
E. S..............	36	27	75.0	9	25.0
J. F..............	16	10	62.5	6	37.5
S. S:.............	15	13	86.7	2	13.3
W. S.*...........	9	6	66.7	3	33.3
A. M............	7	5	71.4	2	28.6
M. F............	6	5	83.3	1	16.7
C. R.*...........	5	5	100.0	0	0.0
A. F.*...........	4	3	75.0	1	25.0
J. B.............	4	3	75.0	1	25.0
H. G............	4	3	75.0	1	25.0
W. B............	4	3	75.0	1	25.0
A. F.*...........	4	2	50.0	2	50.0

* Indicates children.

an individual. Fifty to one hundred per cent of the specimens, from each individual, showed hemolytic Bacillus coli. There was a wide variation in the quantity of the hemolytic type present in the consecutive samples from any one person. The following is representative of the group: March 13, 1933, 0 per cent; March 14, 6 per cent; March 15, 100 per cent; March 16, ·50 per cent; March 17, 74 per cent; March 18, 0 per cent; March 19, 52 per cent. It may be concluded that the greater the number of samples studied, from any one individual, the higher the percentage of persons showing hemolytic Bacillus coli.

Because it is believed by some investigators that the hemolytic Bacillus coli are more likely to be destroyed in the lower bowel by the fermentative organisms and that, consequently, specimens obtained from higher up in the tract give a more accurate picture, 16 stool cultures were examined from 12 volunteers who had taken a cathartic the night before, and the specimen selected was from the last part of the movement. The hemolytic Bacillus coli were present in 9, or 52.3 per cent, of the samples, while in the specimens submitted by these individuals previous to catharsis, the hemolytic Bacillus coli were found in 58.2 per cent.

The hemolytic Bacillus coli colonies showed a variation in the size of the hemolytic zone on blood agar plates, as described by Schmidt. The majority of the strains fell into his " group one." As will be seen later, in the hemolysis test these strains produced a more complete hemolysis of the red cells than did the strains with a narrower hemolytic zone.

Cultural and biological characteristics of the Bacillus coli isolated from the stools

Various tests were carried out on the strains of the hemolytic and non-hemolytic Bacillus coli for their identification (Table III). These tests were performed as soon after the isolation of the strains as possible. The organisms grew well in litmus milk, with the production of acid and clot. On Russell's medium they gave the characteristic appearance with acid and gas in the butt and acid in the slant. None of the strains liquified gelatin at 22° C. The Voges-Proskauer and methyl-red tests were carried out on all of the Bacillus coli isolated—the mediums were prepared according to the method described by Clark and Lubs (8) and the technic followed was as outlined by Levine (9). All of the strains were Voges-Proskauer negative and methyl-red positive, which results conform with the usual reactions for Bacillus coli.

Sugar fermentations. Tests were carried out to determine the action on the various sugars of Bacillus coli strains recovered from stool cultures.

The mediums were prepared and the tests carried out in the following manner:

One per cent peptone and 0.5 per cent sodium chloride were dissolved in a 1.5 per cent agar solution. To this mixture was added 1 per cent of the desired

TABLE III

The results of tests carried out for the identification of the Bacillus coli recovered from stool specimens *

Organism	Number of strains	Gelatin liquefaction	Milk coagulation	Litmus milk			Saccharose	Lactose	Maltose	Glucose	Mannite	Russell's medium		Voges-Proskauer	Methyl-red
				1 day	3 days	12 days						Butt	Slant		
Nonhemolytic B. coli communis...	38	—	+	A	A	A	· O	AG	AG	AG	AG	AG	A	—	+
Nonhemolytic B. coli communior...	6	—	+	A	A	A	AG	AG	AG	AG	AG	AG	A	—	+
Hemolytic B. coli communis.......	28	—	+	A	A	A	O	AG	AG	AG	AG	AG	A	—	+
Hemolytic B. coli communior......	10	—	+	A	A	A	AG	AG	AG	AG	AG	AG	A	—	+

* A indicates acid; AG, acid and gas.

sugar with the exception of maltose. For an indicator, 1 per cent Andrade's solution was used. The medium was tubed in 5 cc. portions and sterilized in the Arnold for 20 minutes on three successive days, with the exception of inulin which was autoclaved for 15 minutes at 15 to 20 pounds pressure. After 24 hours incubation for sterility, the medium was ready for use.

As maltose is disintegrated by heat, it was made up in a 20 per cent solution and filtered through an L5 Pasteur-Chamberland filter. This filtrate was added, under sterile precautions, to the sterilized agar-peptone-salt medium, while warm, in amounts to make a 1 per cent solution, and 5 cc. portions were immediately put into sterilized test tubes, cooled and incubated for 24 hours to test for sterility.

The tubes were then heated in a water bath until the agar medium was melted and then cooled to about 50° C. To the contents of each tube was added 0.2 cc. of a 24-hour broth culture of the Bacillus coli strain; the mixture was shaken carefully and then allowed to cool. A minimum of 4 days incubation was allowed before the final reading was made and the tube discarded, but little change was noted after 48 hours incubation. Positive and negative controls of each sugar as well as uninoculated tubes were included. A result was considered positive for acid when a pink color appeared. The presence of gas was determined by a breaking and cracking of the medium. For most of the saccharose determinations the agar was omitted and Durham fermentation tubes containing 5 cc. of the sugar medium were used. After inoculating and incubating the medium, a bubble in the small inverted tube showed the presence of gas.

All of the strains examined fermented lactose, maltose, glucose, and mannite with the production of acid and gas. Of the 44 nonhemolytic cultures, 6 formed acid and gas in saccharose, and of the 38 hemolytic strains, 10 gave the same reaction. Thus it was demonstrated that the majority of the Bacillus coli strains, regardless of the type, belonged to the Bacillus coli " communis " group.

It was found that 11 strains of Bacillus coli—5 hemolytic and 6 non-hemolytic, which, when first isolated fermented saccharose, later, after several months in the icebox on stock medium, failed to act on the sugar. Conversely, 4 strains that were nonfermenters became fermenters. Examination of all of the strains 6 and 9 months later. showed no further change.

In twelve instances, 5 or more Bacillus coli colonies, either all hemolytic or all nonhemolytic, were picked from an individual stool specimen and tested separately for their action on saccharose medium. In 10 of these, all of the hemolytic or nonhemolytic colonies tested either fermented saccharose or failed to act on it. In the two exceptional cases, the colonies were hemolytic, and both saccharose fermenters and nonfermenters were present in the same specimen. Thus it was found that where both hemolytic and nonhemolytic strains of Bacillus coli are present in the same sample of stool, the reaction to saccharose may be the same or different for the two types of colonies but, in the majority of instances, all of the colonies of each type show the same reaction to the sugar.

Hemolysis tests. The method described by Dudgeon, Wordley and Bawtree (2) was the one employed in the examination of the various strains of the Bacillus coli for their capacity to produce hemolysis of red blood cells. Both rabbit blood and human blood were used, and in the latter instance the tests were carried out twice—the second series was performed six months after the first. Four tubes, two each of the 0.85 per cent and of the 0.5 per cent sodium chloride peptone mediums were inoculated with 0.1 cc. of an 18-hour Bacillus coli culture, and 0.1 cc. of the packed red blood cells was added to one tube of each per cent of saline. All of the tubes were incubated for 24 hours at 37° C. Readings were then taken of the amount of hemolysis present in the tubes containing the red cells. To the contents of the two remaining tubes 0.1 cc. of the packed red cells was added, and the tubes were again incubated for one hour at 37° C. They were then put in the icebox overnight and the degree of hemolysis was noted the following morning.

Altogether 27 strains of Bacillus coli showing hemolysis of the red cells on blood (rabbit) agar plates were tested. The results are shown in Table IV. The red cells of the rabbit blood were hemolyzed more readily than those of the human blood. The organisms possessing active hemolyzing properties showed very little difference in the degree of hemolysis present in the 0.85 per cent and the 0.5 per cent sodium chloride mediums. In the tubes to which human red blood cells had been added before incubation the degree of hemolysis was more marked than in those to which the cells were added the following day. All of the tested strains showed the presence of active hemolyzing properties for the rabbit blood and all but four for the human blood. In these four cases the capacity for hemolysis was present but in a lesser degree.

None of the hemolytic strains showed any loss or diminution of the hemolyzing quality after being kept for six months on artificial medium in

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TABLE IV

*Comparison of the hemolyzing property of the hemolytic Bacillus coli on human and rabbit red blood cells ***

Strain	Human blood								Rabbit blood			
	First series				Second series				24 hours		1 hour	
	24 hours		1 hour		24 hours		1 hour					
	0.85	0.5	0.85	0.5	0.85	0.5	0.85	0.5	0.85	0.5	0.85	0.5
54	C	C	C	C	C	C	C	C	C	C	C	C
53	C	C	C	C	C	C	C	C	C	C	C	C
66	C	C	C	C	C	C	C	C	C	C	C	C
72	C	C	C	C	C	C	C	C	C	C	C	C
48	C	C	C	C	C	C	C	C	C	C	C	C
70	C	C	C	C	C	C	C	C	C	C	C	C
68	C	C	C	C	C	C	C	C	C	C	C	C
49	C	C	C	C	C	C	C	C	C	C	C	C
63	C	C	C	C	C	C	C	C	C	C	C	C
45	C	C	C	C	C	C	C	C	C	C	C	C
59	C	C	C	C	C	C	C	C	C	C	C	C
58	C	C	M	M	C	C	M	M	C	C	C	C
51	C	C	M	M	C	C	M	M	C	C	C	C
69	C	C	M	M	C	C	M	M	C	C	C	C
47	C	C	M	M	C	C	M	M	C	C	C	C
60	C	C	M	M	C	C	M	M	C	C	C	C
69	C	C	M	M	C	C	M	M	C	C	C	C
56	C	C	M	M	C	C	M	M	C	C	C	C
46	C	C	M	M	M	M	T	T	C	C	C	C
61	C	C	T	T	M	M	T	T	C	C	C	C
57	I	I	M	M	I	I	M	M	C	C	C	C
50	M	M	M	M	M	M	M	M	C	C	C	C
65	M	M	M	M	M	M	M	M	C	C	C	C
55	T	T	T	T	T	T	T	T	C	C	M	M
71	T	T	T	T	T	T	T	T	C	C	M	M
52	T	T	T	T	T	T	T	T	C	C	M	M
73	T	T	T	T	T	T	T	T	C	C	M	M

* C: hemolysis of all the red cells. I: hemolysis of nearly all of the red cells. M: distinct coloration of the whole medium. T: slight tingeing of the medium above the red cells. 24 hours: packed red blood cells added at time of inoculation and incubated 24 hours. 1 hour: packed red blood cells added after 24 hours of incubation and incubated an additional hour. 0.85 and 0.5: strengths of sodium chloride in peptone water medium.

the icebox. Also, no change in this capacity resulted from passage through white mice.

Virulence tests. It was a matter of interest to determine the comparative virulence of the hemolytic and the nonhemolytic strains of the Bacillus coli. White mice were used for the tests and the following method was employed:

A 5 cc. tube of broth was inoculated with 0.1 cc. of an 18-hour fresh blood broth growth. This, in turn, was incubated 18 hours, and 0.1 cc. was injected intraperitoneally into a white mouse. If the mouse survived, this procedure was repeated, using a larger dose of 0.3 cc. and a fresh mouse. If the second mouse lived, a larger dose of 0.5 cc. was used; and in this way the doses were increased until a mouse succumbed. *Vice versa*, if a mouse died after the 0.1 cc. injection, smaller doses of 0.05 cc., 0.01 cc., 0.005 cc., or 0.001 cc. were injected until an amount was reached which the mouse was able to survive. All of the mice that died were autopsied, and cultures were made from the peritoneal exudate and heart's blood on blood agar plates.

The test for virulence was carried out on 34 hemolytic and 38 non-hemolytic strains of the Bacillus coli. In Table V is shown, under the various dilutions of the organisms, the minimal lethal doses for the differ-

TABLE V

The comparative virulence for white mice of 34 hemolytic and 38 nonhemolytic strains of Bacillus coli

Minimum lethal doses	Hemolytic Bacillus coli		Nonhemolytic Bacillus coli	
	Number	Per cent	Number	Per cent
cc.				
0.5	0	0	5	13.2
0.3	3	8.8	10	26.3
0.1	9	26.5	4	10.5
0.05	8	23.5	13	34.2
0.01	7	20.6	4	10.5
0.005	7	20.6	2	5.3

ent strains. For example, for 3, or 8.8 per cent, of the hemolytic and 10, or 26.5 per cent, of the nonhemolytic strains, 0.3 cc. was the lowest dilution that killed a mouse. It may be seen that 64.7 per cent of the hemolytic strains and 50 per cent of the nonhemolytic were virulent for mice in doses of 0.05 cc. or less. Thus it is evident that the hemolytic organisms were slightly more virulent than the nonhemolytic.

Agglutination reactions. In order to determine whether there was any biological relationship between the hemolytic and the nonhemolytic strains of the Bacillus coli isolated from the stool cultures, cross agglutination tests were carried out with serums from 8 rabbits immunized against 4 hemolytic and 4 nonhemolytic strains. The 8 immune serums were tested for agglutinins against 40 strains of the Bacillus coli, 22 of which were hemolytic and 18, nonhemolytic.

The agglutination tests were carried out as follows:

A 24-hour broth culture was centrifugalized for 15 minutes and the super-natant fluid removed. The sediment was resuspended in physiological salt solution, making a dilution of one billion organisms per cubic centimeter. The or-

ganisms were killed by heating for one hour at 60° C. On five successive days, rabbits were given intravenous injections of the killed culture in 0.3 cc., 0.6 cc., 0.9 cc., 1.0 cc., and 1.0 cc. doses respectively. After 5 days they were given 5 more injections of 1.0 cc. each of a living 24-hour broth culture. After another 5 days, tests for the agglutination titer were made. All the serum showed a minimum titer of 1:5120 and some went as high as 1:81,920.

The antigens for the agglutination tests were prepared by transferring a loopful of Bacillus coli culture to plain broth and incubating for 18 hours. In this way, a diffuse growth was obtained with every strain. Mixtures of 0.5 cc. of the antigen and 0.5 cc. of the various dilutions of immune serum were made, and the agglutinations were carried out to a titer of 1:5120. A control rabbit's serum, withdrawn before immunization, was tested against the Bacillus coli. Another control consisted of 0.5 cc. of sterile broth and 0.5 cc. of the bacterial antigen. The agglutination tubes were left for two hours in a water bath at 56° C. They were then placed in the refrigerator and readings were made the following morning. The last dilution in which definite clumping of the bacteria could be detected by the naked eye was recorded as the agglutination titer.

The Bacillus coli strains were divided into two main groups, the hemolytic and the nonhemolytic. These, in turn, were separated into two subgroups, the communior and the communis, depending upon their reaction to saccharose. Two immune serums were prepared from each of these four groups, and cross agglutination tests were carried out with 8 immune serums against 12 nonhemolytic communis strains of the Bacillus coli, 6 nonhemolytic communior, 12 hemolytic communis and 10 hemolytic communior.

From Table VI it may be seen that the strains showed no tendency to fall into biological groups. Thus of the 12 strains in the nonhemolytic communis group about 50 per cent of them were agglutinated by immune serum NH–17 and NH–8 of the same group. These same strains were nearly all agglutinated, as well, by serums NH–39 and NH–21, H–55 and H–46, and H–61 and H–62 of the other groups. Three strains were agglutinated by serums NH–8, NH–21, H–46, and H–62 only, and three strains failed to be agglutinated by any of the serums.

The strains in the other three groups showed a marked tendency for cross agglutination similar to that described above for the nonhemolytic communis organisms.

From the point of view of the serums, there was no type specificity. With the exception of serum NH–21, the eight immune serums agglutinated at least half of the strains in all four groups, regardless of type. Serum NH–21 seemed to differ somewhat from the others in that it agglutinated only a few of the strains.

Absorption tests. The nature of the agglutinins in the immune rabbit serums was further studied by means of absorption reactions with the serums of the rabbits which had been immunized with the different types of the Bacillus coli. Each of the 8 serums, with the exception of NH–21, was in turn subjected to absorption tests by eight strains of Bacillus coli, two

TABLE VI

Titer of agglutination reactions with eight serums from rabbits immunized against strains of Bacillus coli, two from each of the four groups

Strain*	Nonhemolytic immune serums				Hemolytic immune serums			
	Communis		Communior		Communis		Communior	
	NH-17	NH-8	NH-39	NH-21	H-55	H-46	H-61	H-62
NH-17 cs....	*1 : 5120*	1 : 5120	1 : 5120	0	1 : 5120	1 : 160	1 : 640	1 : 1280
NH-11 cs....	1 : 2560	1 : 5120	1 : 5120	0	1 : 5120	1 : 320	1 : 2560	1 : 80
NH-5 cs.....	1 : 2560	1 : 5120	1 : 640	0	1 : 2560	1 : 640	1 : 640	1 : 5120
NH-2 cs.....	1 : 160	0	1 : 160	1 : 40	1 : 160	1 : 640	1 : 160	1 : 80
NH-6 cs.....	1 : 80	0	1 : 40	1 : 80	1 : 40	0	1 : 80	1 : 640
NH-4 cs.....	1 : 20	0	1 : 80	0	1 : 80	0	1 : 80	0
NH-3 cs.....	0	1 : 80	0	1 : 640	1 : 5120	1 : 80	0	1 : 80
NH-8 cs.....	0	*1 : 5120*	0	1 : 80	0	1 : 640	0	1 : 5120
NH-1 cs.....	0	1 : 320	0	1 : 640	0	1 : 2560	0	1 : 320
NH-7 cs.....	0	0	0	0	0	0	0	0
NH-9 cs.....	0	0	0	0	0	0	0	0
NH-10 cs....	0	0	0	0	0	0	0	0
NH-39 cr....	1 : 160	1 : 5120	*1 : 5120*	0	1 : 1280	1 : 320	1 : 5120	1 : 1280
NH-33 cr....	1 : 1280	1 : 5120	1 : 2560	0	1 : 640	1 : 320	1 : 160	1 : 5120
NH-35 cr....	1 : 5120	1 : 5120	1 : 2560	0	1 : 80	1 : 320	0	1 : 5120
NH-43 cr....	0	1 : 5120	0	1 : 1280	0	1 : 80	0	1 : 160
NH-21 cr....	0	0	0	*1 : 5120*	0	0	0	0
NH-44 cr....	0	0	0	0	0	0	0	0
H-55 cs.....	1 : 1280	1 : 5120	1 : 2560	0	*1 : 5120*	1 : 5120	1 : 320	1 : 5120
H-49 cs.....	1 : 1280	1 : 5120	1 : 1280	0	1 : 640	1 : 320	1 : 1280	1 : 640
H-58 cs.....	1 : 1280	1 : 5120	1 : 5120	0	1 : 5120	1 : 320	1 : 640	1 : 2560
H-48 cs.....	1 : 640	1 : 5120	1 : 2560	0	1 : 2560	1 : 320	1 : 640	1 : 160
H-51 cs.....	1 : 320	1 : 5120	1 : 2560	0	1 : 1280	1 : 640	1 : 640	1 : 640
H-66 cs.....	1 : 160	1 : 5120	1 : 5120	0	1 : 5120	0	1 : 320	1 : 2560
H-45 cs.....	1 : 80	1 : 40	1 : 40	1 : 80	1 : 40	1 : 80	1 : 40	1 : 80
H-47 cs.....	0	1 : 5120	0	1 : 1280	0	1 : 40	0	1 : 160
H-68 cs.....	0	1 : 5120	0	1 : 2560	0	0	0	1 : 160
H-46 cs.....	0	1 : 1280	0	0	0	*1 : 5120*	0	1 : 640
H-50 cs.....	0	1 : 80	0	1 : 160	0	1 : 320	0	1 : 160
H-65 cs.....	0	0	0	0	0	0	0	0
H-61 cr.....	1 : 1280	1 : 5120	1 : 5120	0	1 : 640	1 : 2560	*1 : 5120*	1 : 1280
H-53 cr.....	1 : 640	1 : 5120	1 : 640	0	1 : 320	1 : 160	1 : 160	1 : 5120
H-67 cr.....	1 : 160	1 : 5120	1 : 320	0	1 : 320	1 : 40	1 : 160	1 : 160
H-59 cr.....	1 : 160	0	1 : 160	0	1 : 160	0	1 : 40	0
H-72 cr.....	0	1 : 5120	0	0	0	1 : 5120	0	1 : 1280
H-62 cr.....	0	1 : 5120	0	0	0	1 : 160	0	*1 : 5120*
H-70 cr.....	0	1 : 80	0	1 : 320	0	1 : 80	0	1 : 40
H-52 cr.....	0	1 : 320	0	1 : 5120	0	0	0	1 : 320
H-63 cr.....	0	1 : 5120	0	1 : 5120	0	0	0	0
H-69 cr.....	0	0	0	0	0	0	0	0

* NH indicates nonhemolytic; H, hemolytic; cs., communis; cr., communior.

from each of the four groups that had shown an agglutination titer of 1:1280 or higher. Agglutination tests were then carried out on each of the absorbed serums, using for antigen the absorbing strains. A second agglutination test was carried out with each absorbed serum, using for antigen the strain with which the rabbit was inoculated to produce that serum.

The method used for carrying out the tests was as follows:

The sediment from 75 cc. of a 24-hour broth culture of a strain of Bacillus coli which was found to be the dose factor necessary to completely remove the immune bodies for the homologous strain was mixed with 0.2 cc. of a 1:10 dilution of the immune serum. The tube was shaken and placed in a water bath at 37° C. for two hours, during which time it was agitated at frequent intervals. It was then placed in an icebox overnight. The following morning the mixture was centrifugalized and the supernatant fluid removed. Agglutination tests were set up, using the same dilutions as in the original tests. Control agglutinations were carried out with unabsorbed serum. All of the tubes were placed in a water bath at 56° C. for two hours and, after standing in the icebox overnight, readings were made.

As may be seen from Table VII, in the cases where agglutination tests were carried out with absorbed serum, using the absorbing strain of the Bacillus coli as antigen; complete absorption occurred in every case. However, when the homologous strain for each serum was used there was either slight or no absorption of the agglutinating bodies. Thus the immune serums contained agglutinins common to many of the strains but these were not identical with those for the homologous strains.

DISCUSSION AND SUMMARY

One hundred and sixty-nine specimens of stools from 73 healthy individuals were cultured for the presence of the hemolytic Bacillus coli and this organism was found in 114, or 67.4 per cent, of the samples.

The results obtained from the study of single and repeated cultures from the same person were of particular interest. In the 73 first or single specimens cultured the incidence of hemolytic Bacillus coli was 56.2 per cent. In the 25 cases where two or more specimens from the same individual were studied the figure rose to 88 per cent, and in 12 instances, where four or more cultures were examined, hemolytic Bacillus coli were recovered in 100 per cent of the subjects. From these figures it seems reasonable to conclude that the hemolytic Bacillus coli may be recovered from practically every healthy individual, and is a normal inhabitant of the intestinal tract, while for an accurate estimate of the type of Bacillus coli present several specimens should be examined.

All of the hemolytic and nonhemolytic strains of the Bacillus coli isolated conformed to the usual cultural reactions. The majority of the strains, both hemolytic and nonhemolytic, failed to ferment saccharose and

thus were classed in the Bacillus coli "communis" group. A few of the strains which fermented saccharose when first isolated later, after several months in the icebox, failed to act on the sugar.

All of the hemolytic strains showed active hemolyzing properties for the red cells of rabbit blood and all but four for the red cells of human blood. These latter strains were of the type that showed hemolysis only beneath the colony on blood (rabbit) agar plates.

TABLE VII

Titer of agglutination reactions with serums from seven immunized rabbits before and after absorption of agglutinins

Immune serums*	Absorbing strains	Bacterial antigens							
		NH-17	NH-39	H-55	H-61	NH-5	NH-33	H-53	H-58
NH-17	NH-17	0							
	NH-39	1:40	0						
	H-55	1:640		0					
	H-61	1:320			0				
	NH-5	1:1280				0			
	NH-33	1:640					0		
	H-53	1:320						0	
	H-58	1:40							0
	Unabsorbed	1:5120	1:160	1:1280	1:1280	1:2560	1:1280	1:640	1:1280
NH-39	NH-17	0	1:1280						
	NH-39		0						
	H-55		1:640	0					
	H-61		1:640		0				
	NH-5		1:320			0			
	NH-33		1:1280				0		
	H-53		1:320					0	
	H-58		1:40						0
	Unabsorbed	1:5120	1:5120	1:2560	1:5120	1:640	1:2560	1:640	1:5120
H-55	NH-17	0		1:2560					
	NH-39		0	1:640					
	H-55			0					
	H-61			1:1280	0				
	NH-5			1:640		0			
	NH-33			1:160			0		
	H-53			1:160				0	
	H-58			1:1280					0
	Unabsorbed	1:5120	1:1280	1:5120	1:640	1:2560	1:640	1:320	1:5120
H-61	NH-17	0			1:640				
	NH-39		0		1:1280				
	H-55			0	1:160				
	H-61				0				
	NH-5				1:320	0			
	NH-33				1:160		0		
	H-53				1:160	·		0	
	H-58				1:160				0
	Unabsorbed	1:640	1:5120	1:320	1:5120	1:640	1:160	1:160	1:640

TABLE VII (continued)

Immune serums	Absorbing strains	Bacterial antigens							
		NH-8	H-46	H-62	NH-1	NH-35	H-55	H-72	NH-33
NH-8	NH-8	0							
	H-46	1 : 320	0						
	H-62	1 : 1280		0					
	NH-1	1 : 160			0				
	NH-35	1 : 640				0			
	H-55	1 : 1280					0		
	H-72	1 : 320						0	
	NH-33	1 : 2560							0
	Unabsorbed	1 : 5120	1 : 1280	1 : 5120	1 : 320	1 : 5120	1 : 5120	1 : 5120	1 : 5120
H-46	NH-8	0	1 : 5120						
	H-46		0						
	H-62		1 : 160	0					
	NH-1		1 : 1280		0				
	NH-35		1 : 80			0			
	H-55		1 : 1280				0		
	H-72		1 : 1280					0	
	NH-33		1 : 80						0
	Unabsorbed	1 : 640	1 : 5120	1 : 160	1 : 2560	1 : 320	1 : 5120	1 : 5120	1 : 320
H-62	NH-8	0		1 : 5120					
	H-46		0	1 : 160					
	H-62			0					
	NH-1			1 : 40	0				
	NH-35			1 : 1280		0			
	H-55			1 : 2560			0		
	H-72			1 : 640				0	
	NH-33			1 : 1280					0
	Unabsorbed	1 : 5120	1 : 640	1 : 5120	1 : 320	1 : 5120	1 : 5120	1 : 1280	1 : 5120

* NH indicates nonhemolytic; H, hemolytic.

Conflicting results concerning the comparative virulence of the hemolytic and the nonhemolytic strains of the Bacillus coli recovered from stools appear in the literature. In the present study 35 per cent of the hemolytic strains and 50 per cent of the nonhemolytic strains required 0.1 cc. or more of the culture to kill the mouse, while 65 per cent of the hemolytic and 50 per cent of the nonhemolytic required 0.05 cc. or less of the culture. Although these results show that the hemolytic organisms are somewhat more virulent for mice than are the nonhemolytic the difference is not sufficiently striking to be important.

In the present investigation the Bacillus coli recovered from the stool specimens fell into four groups: hemolytic communior, hemolytic communis, nonhemolytic communior, and nonhemolytic communis. It was a matter of interest to determine the immunological relationship between the various strains of each type, as well as the biological characteristics common to the four groups.

For the production of immune serum, rabbits were inoculated with hemolytic and nonhemolytic strains of Bacillus coli, 2 from each of the four groups. Contrary to the findings of other investigators (2, 3), the nonhemolytic strains produced immune bodies in the rabbits just as readily and to as high a titer as did the hemolytic. This may be due to the fact that a different method of immunization was employed in this study.

When a representative number of strains from each type was selected and agglutination and absorption tests were carried out, the results proved interesting. A high percentage of the strains of each group were agglutinated at least in some degree by the immune serums of the other three groups. Thus a biological relationship seemed to exist not only among the members of each group but also between the strains of all four groups, regardless of their type. When absorption tests were carried out on each immune serum with strains showing a high titer of agglutination, the agglutinins for the absorbing strain were completely removed. However, when the homologous strain for the absorbed serum was used as antigen, it was found that there had been only slight or partial absorption of the agglutinins for the test strain. Thus it was found, in the majority of instances, that the strains of the Bacillus coli, regardless of the presence or absence of hemolysis, and of their reaction to saccharose, had antigenic properties in common, although no strains were found that seemed to be definitely homologous.

CONCLUSIONS

1. For an accurate determination of the presence of the hemolytic Bacillus coli in the stool of an individual several specimens should be examined.

2. The hemolytic Bacillus coli is a normal inhabitant of the intestinal tract of healthy individuals.

3. The hemolytic strains of Bacillus coli recovered from stool specimens were found to be only slightly more virulent for white mice than were the nonhemolytic.

4. The Bacillus coli recovered from stool cultures appear to be heterologous strains having agglutinins more or less in common.

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THE RELATION OF THE CEREBROSPINAL AND VENOUS PRESSURES IN HEART FAILURE

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Elevation of pressure in the intracranial veins immediately results in rise in the tension of the cerebrospinal fluid. This is well illustrated by the familiar Queckenstedt maneuver, in which compression of the jugular veins is followed by increase in cerebrospinal pressure. Since the cerebrospinal tension returns to its previous level as soon as the jugular compression is discontinued, the rise is obviously due to the engorgement of the intracranial tributaries of these veins. Weed and Hughson (1) have shown in animal experiments that alteration in intracranial venous pressure results in a change in the pressure of the cerebrospinal fluid in the same direction but of less magnitude. It is not only in abrupt and transitory engorgement of the intracranial veins that the tension of the fluid rises. Similar hypertension of the cerebrospinal fluid has been observed in mediastinal tumor and tuberculous mediastinitis (Porot (2)), as well as after deep jugular ligation (Guillain (3)). One would therefore anticipate that venous engorgement due to insufficiency of the right heart would likewise be accompanied by elevation in the tension of the cerebrospinal fluid. That such is actually the case has been found by Tzanck and Renault (4), Lamache (5), and Harrison (6).

In this communication, we desire to present comparative measurements of the venous and cerebrospinal pressures during the course of heart failure. Observations on the pressure changes resulting from removal of cerebrospinal fluid in patients with heart failure will also be described.

METHODS

The pressure of the cerebrospinal fluid was measured in the lumbar subarachnoid space with the usual water manometer, the patient being relaxed in the lateral position. Care was taken to avoid loss of fluid before the measurement and to allow time for the system to attain equilibrium. Normal values range between 8 and 18 cm. of water. To study the pressure changes resulting from withdrawal of fluid, a three-way stopcock was attached to the needle and to the manometer, the fluid being drained through the third orifice. The pressure was measured after each cubic centimeter withdrawn.

The venous pressure was measured by a direct method in a large ante-cubital vein level with the right auricle, an L-tube of glass being used for a manometer. With this technic, the venous pressure in health is between 4 and 8 cm. of water.

OBSERVATIONS

In Figure 1 are plotted 85 measurements of venous and spinal pressures in 55 patients with various types of heart disease, including the rheumatic, syphilitic, hypertensive and arteriosclerotic. In each patient at least one measurement was made while manifestations of decompensation were pres-ent, but there are also included the observations after compensation had returned.
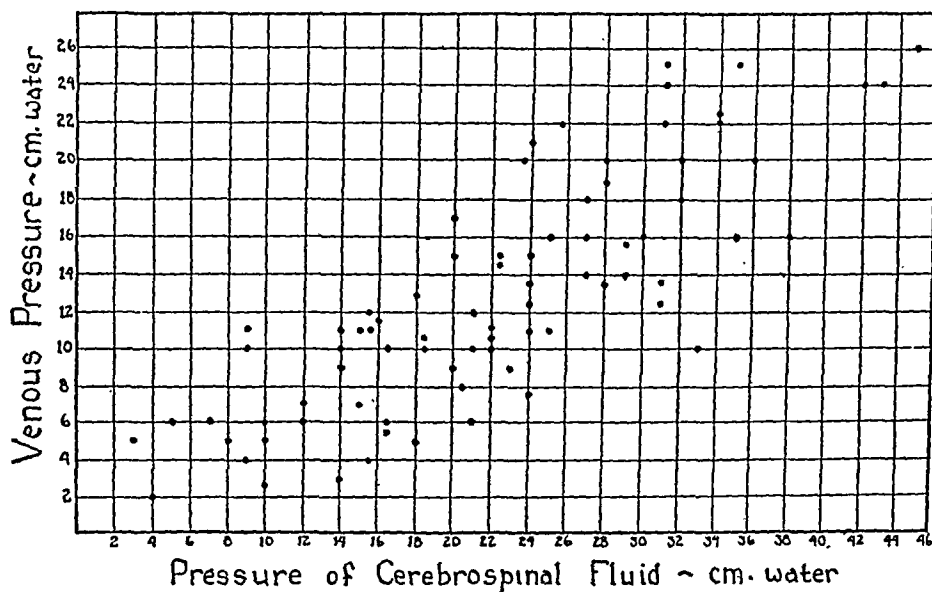
FIG. 1. VENOUS AND CEREBROSPINAL PRESSURES IN 55 PATIENTS
WITH HEART FAILURE

Inspection of Figure 1 reveals that there is a well marked correlation between the venous and cerebrospinal pressures in the course of heart fail-ure. Both pressures obviously tend to parallel variation, although the marked divergences from a strictly linear distribution show that there is no constant ratio between the two variables, and that the venous pressure is only one of the factors, albeit an important one, which determine the cere-brospinal pressure.

The influence on the cerebrospinal pressure of changes in venous pres-sure during the course of heart failure is better brought out by serial ob-servations on the same patient, comparing the changes in venous pressure with those in cerebrospinal tension. We have made a number of such ob-servations, some of which are summarized in Table I.

TABLE I

Serial observations of venous and cerebrospinal pressures

Case number	Diagnosis	Date	Cardiac status	Venous pressure	Cerebro-spinal pressure
				cm. water	*cm. water*
49539	Rheumatic heart disease	July 10, 1932	Moderate failure	14	29
		July 18, 1932	Improved	9	20
		July 25, 1932	Improved	9	14
		August 2, 1932	Improved	5	8
Re-admission		November 20, 1932	Moderate failure	22	25
47919	Rheumatic heart disease	June 3, 1932	Severe failure	24	31
		July 8, 1932	Improved	6	21
		July 15, 1932	Improved	7	15
58353	Rheumatic heart disease	October 9, 1933	Severe failure	23	43
		October 12, 1933	Improved	10	20
		October 17, 1933	Increased failure	18	32
		November 9, 1933	Improved	10	22
49523	Hypertensive and arteriosclerotic heart disease	July 11, 1932	Mild failure	11	24
		July 16, 1932	Improved	4	9
50093	Arteriosclerotic heart disease	August 2, 1932	Moderate failure	11	25
		August 8, 1932	Improved	6	16
47423	Hypertensive heart disease	April 26, 1932	Moderate failure	12	21
		May 3, 1932	Improved	3	14
49617	Rheumatic heart disease	July 13, 1932	Moderate failure	20	23
		July 18, 1932	Improved	6	12

From the observations recorded in Figure 1 and Table I, it is clear that as the venous pressure rises in consequence of right heart failure the spinal pressure also mounts. However, even in the same patient there is no constant ratio between the venous and cerebrospinal pressures. Nevertheless, one relationship is very striking; namely, that the spinal pressure is greater in 81 of a total of 85 measurements than the venous pressure, and in the exceptions the difference is so small as to be within the limits of error of the methods used. It will be recalled that the same general relation holds in health, i.e., the pressure in the lumbar subarachnoid space is higher than that in the peripheral systemic veins.

The highest spinal pressure that we observed in an uncomplicated instance of heart failure was 45 cm. of water in a patient with venous pressure of 26 cm. However, it is to be presumed that decidedly higher spinal ·pressure may result from heart failure, for in unusual instances the venous pressure may exceed 35 cm. We did not measure the spinal pressure in any such cases because of the poor general condition of the patients.

When the venous pressure falls as a result of improvement of the heart, the tension of the spinal fluid also drops. However, our observations indicate that there is often a lag in the fall of spinal pressure behind that of venous pressure, so that spinal pressure may still be definitely elevated for several days after venous pressure has returned to normal. The significance of this lag will be discussed below.

The elevation of the tension of the cerebrospinal fluid persists as long as venous engorgement is present. In protracted right heart failure the tension of the fluid may remain over 30 cm. of water for weeks or even months.

Elevation of the tension of the spinal fluid due to heart failure apparently occurs only through the intermediacy of venous engorgement, for in pure left ventricular failure with normal systemic venous pressure we observed normal tension in the subarachnoid space even though the lungs were engorged and dyspnea was severe.

DISCUSSION

The foregoing observations show that elevation in venous pressure due to failure of the right heart results in a rise in the tension of the cerebrospinal fluid. The question then arises of the mechanism through which the rise in systemic venous pressure results in hypertension of the cerebrospinal fluid. Three main factors would appear to be concerned:

1. Engorgement of the intracranial (and intraspinal) veins, which tends to raise the intracranial tension because of the unyielding cranium. That the intracranial veins are engorged in failure of the right heart is indicated during life by the ophthalmoscopic appearance of the retinal veins. Moreover, since the increased pressure in the antecubital vein which is actually observed is a consequence of higher pressure in the superior vena cava, the pressure in the intracranial veins must also be elevated, since they also drain into the superior vena cava. There can therefore be no doubt that engorgement of the intracranial and intraspinal veins is an important, probably usually the most important, cause of the increased pressure of the cerebrospinal fluid in right heart failure. However, that it is not the only factor is shown by the above mentioned observation that as the patient recovers from insufficiency of the right heart and the venous pressure returns to normal, the tension of the cerebrospinal fluid usually falls more slowly so that there is a stage in which cerebrospinal pressure is still elevated despite normal venous pressure.

2. Increase in the bulk of the brain substance and meninges because of edema due to right heart failure. That this actually occurs, though in widely varying degree, is well known from postmortem observations.

3. Increase in the volume of cerebrospinal fluid, i.e., hydrocephalus. That this factor operates is suggested by the widely held conception that the physical mechanisms of filtration and diffusion are concerned both in the formation and the resorption of cerebrospinal fluid. Since increase in venous pressure entails augmentation in capillary pressure, it might be thought *a priori* that filtration is aided and resorption hindered, with resultant increment in the volume of cerebrospinal fluid. On the other hand, the engorgement of the intracranial blood vessels and the edema of the brain substance tend to diminish the size of the ventricles and thus oppose the accumulation of cerebrospinal fluid.

In order to obtain information regarding the volume of cerebrospinal fluid in patients with heart failure, the effect of withdrawal of the fluid on spinal pressure was studied. It has been found by Ayala (7) that the larger the volume of cerebrospinal fluid, the smaller the drop in pressure resulting from the removal of a given volume of fluid. Ayala has expressed this finding in a formula, by the application of which to the pressure changes resulting from removal of cerebrospinal fluid, he has been able to differentiate the rise in lumbar pressure due to increase in volume of the brain substance (e.g., brain tumor) from that due to increase in the volume of cerebrospinal fluid (e.g., serous meningitis). We have plotted in Figure 2 the pressure changes resulting from removal of cerebrospinal fluid in 15 cardiac patients. According to Ayala's formula, the larger the initial volume of cerebrospinal fluid, the less the slope of the line through the pressures after successive removals of fluid. On the other hand, a small initial volume of fluid would be characterized by a rapid drop in the line of pressures. Inspection of Figure 2 reveals that, in a general way, the lines of the different patients run parallel courses at levels determined largely by the initial cerebrospinal pressure and consequently by the venous pressure. This would seem to be significant, though indirect, evidence that there is no marked change in the volume of cerebrospinal fluid in patients with heart failure.

From these findings, it would appear that the increased cerebrospinal pressure in right heart failure is due principally to the engorgement of the intracranial and intraspinal vessels, but that edematous swelling of the nervous tissue and meninges also plays some part.

Symptoms. It is remarkable that, despite the marked and often protracted elevation of intracranial tension due to right heart failure. our patients did not exhibit symptoms definitely referable to the increased cerebrospinal pressure. Even those with lumbar pressures of between 30 and 45 cm. of water rarely complained of headache. Apart from those with malignant hypertension, only one of the patients had slight papilledema. and

he had the contributing factor of high grade myopia with its associated decrease in intraocular tension. One of us has observed an instance of severe right heart failure secondary to pulmonary disease in which there were papilledema and retinal hemorrhages that cleared up with the other mani-
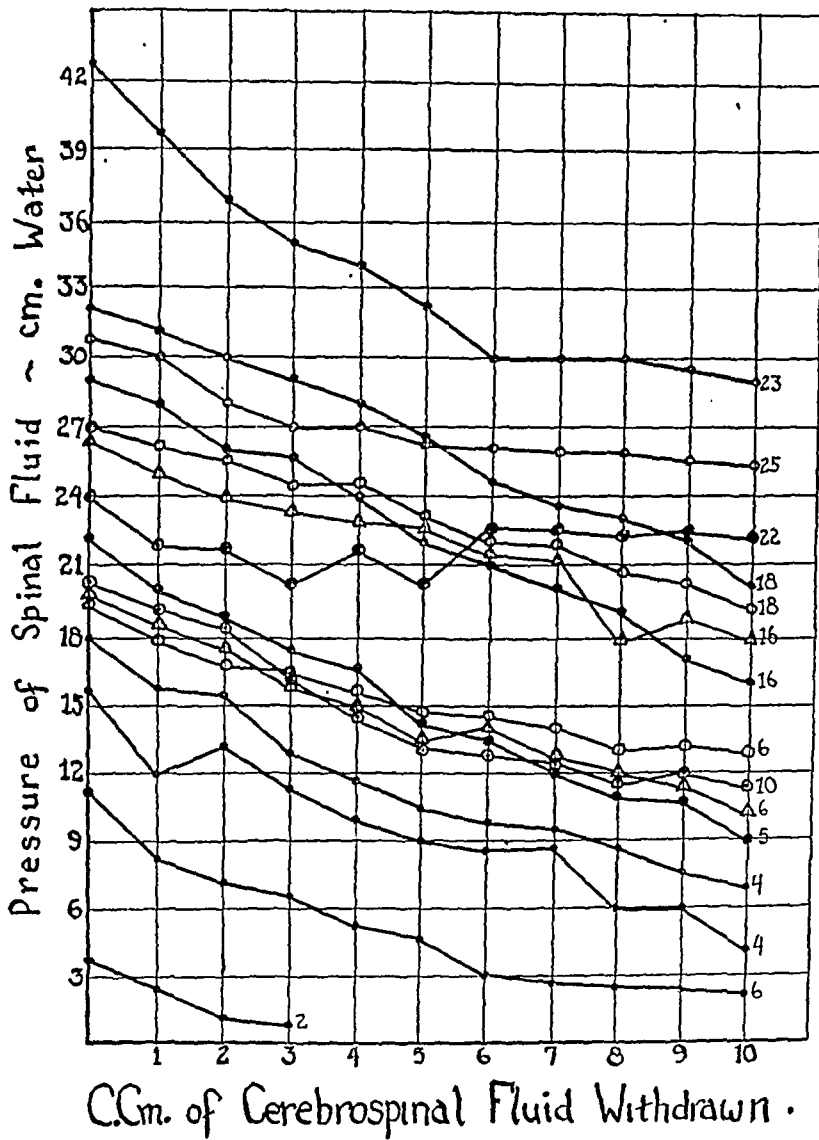
FIG. 2. EFFECT OF WITHDRAWAL OF CEREBROSPINAL FLUID ON THE PRESSURE IN THE LUMBAR SUBARACHNOID SPACE

The figure at the right of each line is the venous pressure just before the lumbar puncture.

festations of cardiac insufficiency when digitalis was administered. But in view of the findings in this series of cases, the ocular changes would appear to have been due, in part at least, to factors other than increased intracranial tension.

SUMMARY

Elevated venous pressure due to failure of the right heart is accompanied by increase in the tension of the cerebrospinal fluid. The cerebrospinal pressure is almost always, if not always, higher than the venous pressure. When the venous pressure falls as a result of improvement of the heart, the cerebrospinal pressure also falls, although often there is a lag behind the drop in venous pressure.

The cerebrospinal pressure is not elevated in left heart failure with normal venous pressure.

The increased cerebrospinal pressure in right heart failure is due principally to engorgement of the intracranial and intraspinal vessels, but edematous swelling of the nervous tissues and meninges also participates. No evidence was obtained that the volume of cerebrospinal fluid is increased.

Symptoms due to increased intracranial tension were not observed, although the spinal fluid pressure rose as high as 45 cm. of water.

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THE RELATION OF RHEUMATIC FEVER TO POSTSCARLA-
TINAL ARTHRITIS AND POSTSCARLATINAL HEART
DISEASE—A FAMILIAL STUDY [1]

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INTRODUCTION

The relationship which postscarlatinal arthritis and postscarlatinal heart disease may bear to rheumatic fever has for many years been an unsettled question. Furthermore, since there is no exact information as to the nature of these sequelae of scarlet fever, nor of the nature of rheumatic fever, it is obviously difficult to compare them. It should be possible, however, to say whether or not fundamental similarities, or differences exist between the familial conditions under which these two syndromes arise, and it is with such a comparison that this paper will be concerned.

Postscarlatinal rheumatism and heart disease—A brief clinical review. The syndrome of postscarlatinal nonsuppurative arthritis or rheumatism with which carditis may or may not be associated, represents an entity so well-known that it hardly merits a review of its clinical features. However, some statements concerning the type, and incidence of these scarlet fever complications seem necessary. Various types of lesions may of course involve either the joints or the heart during or following scarlet fever; the lesions of the joints may be either suppurative or nonsuppurative, and the cardiac lesions may be classified, according to Swift (1), into three groups: viz., (i) toxic lesions which generally occur early in the disease; (ii) septicopyemic lesions which may appear early or late; and (iii) so-called allergic lesions which usually appear late in the disease. Our use of the terms postscarlatinal arthritis and postscarlatinal carditis refers solely to the nonsuppurative arthritis and to the so-called allergic carditis, respectively, both of which arise ordinarily during the secondary phase of scarlet fever. When present, this secondary phase, the frequency and importance of which was first emphasized by Schick (2), appears after an interval of from 7 to 30 days following the primary, exanthematous or toxic phase of scarlet fever. If the picture is not confused by the presence of suppurative complications, or of serum disease, the secondary phase (which frequently may be quite mild and of but a few days' duration) generally becomes manifest by recurrence of fever, moderate soreness of the throat and enlargement of some of the lymph nodes. It is usually at this time that in addition to the symptoms just mentioned, arthritis, carditis or acute hemorrhagic nephritis may also appear.

Postscarlatinal nonsuppurative arthritis has been roughly divided into two groups on the basis of severity, the severer grades having been termed "rheumatism," and the more common, milder forms "serous synovitis." Both forms may simulate the arthritis seen in rheumatic fever, the serous type recalling in particular the mild forms of arthritis or periarthritis characteristic of juvenile rheumatic fever. Arthritis of both types is said to occur in about 6 to 10 per cent of all cases of scarlet fever (3, 4). The incidence seems to be greater in some epidemics of scarlet fever than in others and to increase with the age of the patient. Thus, according to Weaver's statistics (3), only 2.2 per cent of patients develop postscarlatinal arthritis in the first decade of life, whereas the incidence is 4.5 per cent in the second decade, and 13.3 per cent in the third.

Estimates with regard to the usual per cent of patients who develop residual heart disease following scarlet fever are much more difficult to obtain. Convincing data of this kind are scanty because they should be derived from large series of scarlet fever cases which have been followed for at least one or two years. The importance of prolonged observation is based upon the fact that the so-called allergic type of postscarlatinal carditis may become manifest rapidly or not until many months have elapsed, which is again similar to the manner in which indisputable rheumatic carditis develops following an upper respiratory infection.

Nevertheless, many estimates on the incidence of various cardiac lesions during or following scarlet fever have been reported and warrant mentioning. Transient cardiac murmurs appear frequently during the acute or early convalescent stage of scarlet fever, and are said to occur in from 18 to almost 50 per cent of the cases (5). The significance of such murmurs is unknown, although when accompanied by abnormalities of the pulse most of them have been considered as manifestations of actual, although temporary, myocardial damage of the so-called toxic type. Evidences of myocarditis of this and other forms have been described in about 5 per cent of cases of scarlet fever (6). As for the incidence of endocarditis it has generally been placed at less than 0.5 per cent (6, 7, 8). If this is correct postscarlatinal heart disease differs from rheumatic heart disease in at least one respect, namely, that in clinical rheumatic heart disease endocarditis is present in the great majority of cases.

As for the association between carditis of any kind and arthritis following scarlet fever, the great difference between the total incidence of the two, has led to the belief that such an association is infrequent. This conception is held in spite of the fact that the converse of the above situation is altogether different, in that it has been stated that about half the patients with postscarlatinal endocarditis or pericarditis have suffered also from postscarlatinal arthritis (9). It is in evaluating such data that we would again point out that owing to the lack of adequate follow-up studies upon patients who have sustained postscarlatinal arthritis there is little information as to the number who eventually develop carditis.

Some have considered postscarlatinal rheumatism and so-called allergic carditis to be more or less specific manifestations or complications of scarlet fever, though perhaps distinct from the toxic lesions of the disease. Their views find expression in the majority of text-book articles on scarlet fever which we have consulted. They agree, however, that if a patient, who has previously had rheumatic fever, sustains an attack of scarlet fever, the chances of his developing postscarlatinal arthritis are greatly enhanced, and under these circumstances the clinical picture more nearly corresponds

to that of rheumatic fever. Others do not recognize this distinction and believe that the majority of cases of postscarlatinal rheumatism and carditis, with or without a previous history of rheumatic fever, may be better regarded as rheumatic fever "activated" perhaps by scarlet fever in the same manner in which other streptococcus infections are prone to do this in a patient who either has "latent" rheumatic fever, or (for want of a better term) a rheumatic diathesis (10, 11, 12, 13).

The major purpose of this study is to test the adequacy of the two views above quoted, by approaching the problem from the standpoint of familial epidemiology. Experience has shown rheumatic fever to be a disease in which the familial incidence is high (14, 15, 16). Consequently it has seemed important to determine whether or not this high incidence of rheumatic fever exists in the families of those who sustain postscarlatinal arthritis or carditis. Such an investigation should also shed some light on the circumstances under which these sequelae of scarlet fever develop. Our aim therefore is to answer the question as to whether the acquisition of arthritis or carditis following scarlet fever is ubiquitous, or whether it represents the manner in which a patient with familial "rheumatic tendencies" may react to scarlet fever.

METHODS

The family approach. The idea of considering the family as a unit through which disease may spread is a concept which has proved of increasing value in the study of human disease. Its value is based on the fact that in the family, common hereditary and environmental conditions exist in a group of individuals living in intimate contact with one another, who are generally quite conscious of their group life. In studies on the spread of tuberculosis within families, this approach has been emphasized by Opie and his collaborators (17), and it is to the latter work we are particularly indebted for our methods. In a previous study by two of us (18) on the spread of rheumatic fever through families a more detailed outline of the methods which are used in the present paper has been presented.

Source of families and methods of study employed. The patients whose families were enrolled in this study were drawn from the Pediatric and Medical wards, and from the Dispensary Clinics of the New Haven Hospital. All of them lived in the City of New Haven or its environs. It was our object to restrict the group to some extent to those families which had utilized the Dispensary as their "family doctor," and whose members had visited it from time to time for trivial illnesses as well as those of a more serious nature. The records of some of them covered a period of twenty years. Occasionally it was necessary to draw upon the records of other hospitals or of private physicians to fill certain gaps.[2] Most of the families described in this paper, including the control groups, were selected at some time between 1928 and 1932. Subsequent to their selection they were visited at least once a year and, at the time of the visit,

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histories were taken and all available members were examined. If any member of the family happened to be under the care of a practicing physician, the physician was either seen personally or called on the telephone in order to explain our reason for questioning or examining his patient.

We believe that this type of family study is necessary if one is to determine even with rough accuracy the familial incidence of rheumatic fever. It is not enough to ask a parent or child whether other members of the family have had " rheumatism," " St. Vitus dance " or "heart disease," or even scarlet fever, for the information thus obtained is apt to be very different from that obtained by a personal talk with each member, a physical examination with particular attention to the heart, and a careful perusal of his or her previous medical records when available. We also believe that the value of such determinations of familial incidence is greatly enhanced by the inclusion of a reasonable number of control " non-rheumatic " families.

In the course of the work the assistance of social workers and members of the New Haven Visiting Nurse Association have been utilized for making appointments and for gathering non-medical data, but all visits for ascertaining data on the health of the family were made by one of us.

Nomenclature and diagnostic criteria. The term *rheumatic fever* hardly requires definition, but has been employed by us to designate any of the manifestations which, we believe, have represented a period of activity of the disease in question. Thus acute or subacute arthritis, active endocarditis, myocarditis, and pericarditis, Sydenham's chorea, otherwise unexplained fever and malnutrition, frequent nose-bleeds, etc., have all under certain circumstances been considered as manifestations of active rheumatic fever in this report.

The term *postscarlatinal arthritis* or *rheumatism*, as previously stated, has been employed to designate those examples of nonsuppurative arthritis in which joint pains of moderate severity, with or without demonstrable swelling of the joints, occurred over a period of several or more days, and began within one to four weeks following the onset of scarlet fever. Similarly in the diagnosis of *postscarlatinal carditis* we have included those cases in which active valvular, myocardial or pericardial lesions of the heart (exclusive of acute bacterial endocarditis or suppurative pericarditis) were manifest within a similar period from the onset of scarlet fever. Cases in which the only evidence of possible myocarditis consisted in the presence of transient murmurs, which may or may not have been accompanied by a brief period of tachycardia, were not included; nor were those cases included which did not develop clear-cut cardiac lesions until months after their attack of scarlet fever.

Charting results. A chart was designed for each family similar to the type which has been used routinely during the past five years for recording data on patients admitted to the Medical Rheumatic Fever Clinic and the Pediatric Cardiac Clinic of the New Haven Dispensary. The type of chart employed, together with a brief explanatory note is shown in Figure 1. It is constructed to represent the life history of the family, and the time relationships within the family between incidents with which we have been concerned.

Selection of families. Four groups of families were selected.

Group A. *Control* families chosen because one of the members, who did not present any signs of having had rheumatic fever or postscarlatinal rheumatism or carditis, and did not give a history of having had any of the manifestations of these conditions, was attending either the General, or one of the special Pediatric Clinics. This group totalled 16 families; 9 were chosen from the
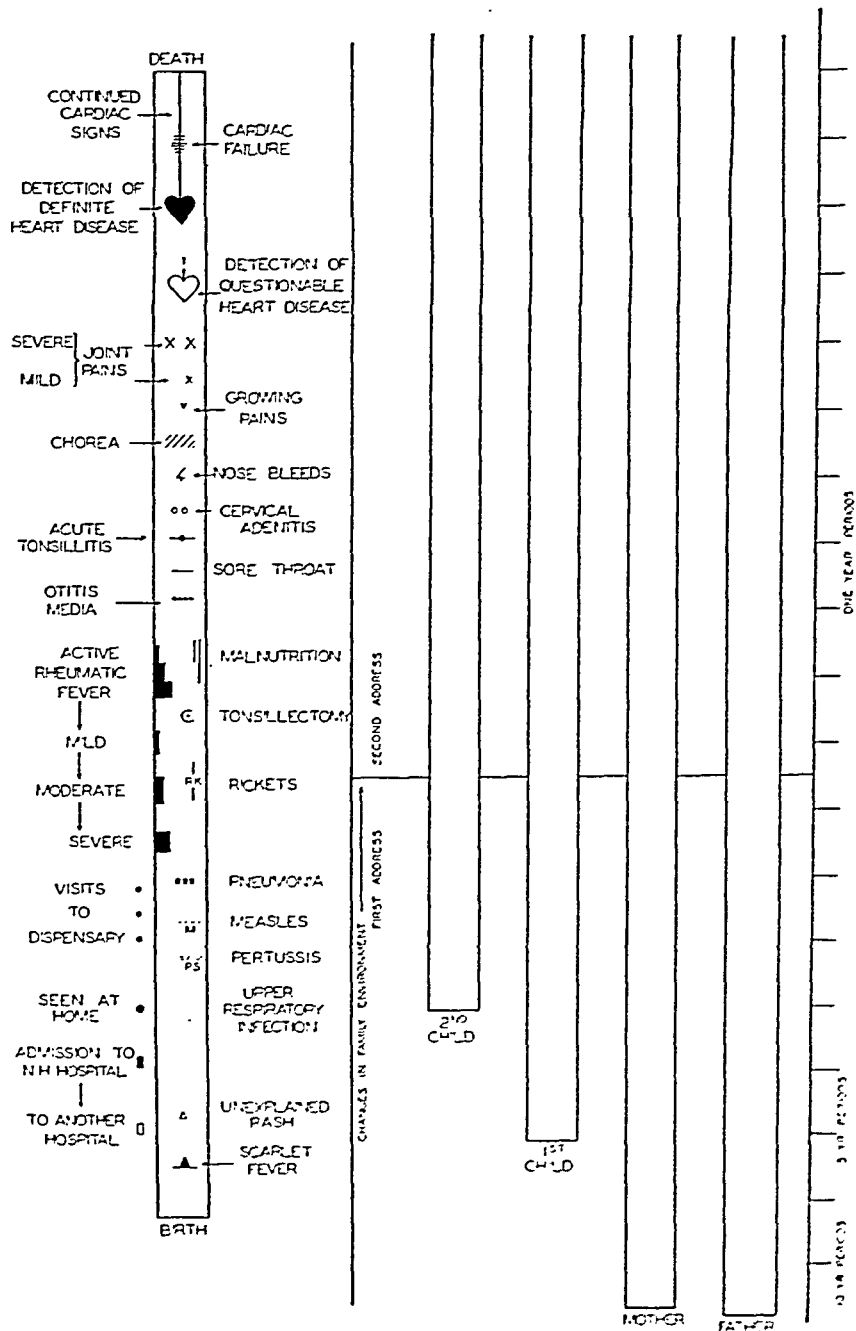
FIG. 1. CHART FOR RECORDING FAMILY DATA

On the right of the figure the age of the group starting with the birth of the parents has been marked in periods of years. The life span of each individual is designated by vertical columns enclosing a space for the chronological tabulation of illnesses and other pertinent events. On the left of the figure are shown the legends for designating these illnesses and events.

General Clinic, 4 from a Clinic for mentally defective or backward children, and 3 from the Syphilis Clinic.[3]

Group B. *Control scarlet fever* families chosen because one of the members, attending the General Pediatric Clinic, had not had rheumatic fever but was known to have had scarlet fever without a subsequent attack of rheumatism or carditis. A special effort was also made in the selection of this group to include families in which several cases of scarlet fever had occurred besides the one which represented the basis on which the family was chosen.

Group C. *Rheumatic* families, chosen because one of the members either had, or was known to have had, one of the manifestations of rheumatic fever, the onset of which did not follow an attack of scarlet fever.

Group D. *Postscarlatinal rheumatism or carditis* families, chosen because in one of the members the onset of rheumatism or carditis had followed immediately upon an attack of scarlet fever.

The families were also chosen so that the ages of individual members in each of the four groups would be about the same. Thus in all groups about 70 per cent represented individuals under 20 years of age, and 40 per cent under 10 years.

From the family charts (similar to those shown in Figures 2 and 3) the incidence of various manifestations of rheumatic fever was determined in the case of members other than the one who represented the basis on which the family had been selected. Of course in assembling these incidence determinations no examples of frank postscarlatinal rheumatism or carditis were listed as examples of rheumatic fever.

RESULTS

The rheumatic background of individuals who develop postscarlatinal rheumatism or carditis. Our aim, as already stated, has been to obtain a more adequate past and family history from patients who have sustained postscarlatinal rheumatism or carditis, by going to the patient's family and by determining whether or not a high incidence of the manifestations of rheumatic fever could be detected in the other members. An interpretation of this incidence determination can be made only when it is compared with similar incidence determinations from the control groups. These appear in Table I. In the control group A the total incidence of individuals who at any time in their life had shown manifestations of rheumatic fever, was 4.3 per cent. This is a little higher than the incidence (2.9 per cent) found by Faulkner and White (15) in a group of normal families in which an intensive search for the manifestations of rheumatic fever was also made. A considerably higher familial incidence of rheumatic fever manifestations (12 per cent) was found in our control scarlet fever families (Group B). One possible explanation of the high figure in this group is

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[3] Originally included in this group were a number of families selected from the Tuberculosis Clinic. Somewhat to our surprise we found a high incidence of rheumatic fever in these so-called tuberculous families, quite out of proportion to the incidence in the other control families. We believe this fact deserves further study and for that reason the attempt will not be made to include them in this paper.

TABLE I

The rheumatic background of individuals who develop postscarlatinal rheumatism or carditis

Type of family	Number of families	Number of individuals*	Per cent of those examined showing:			
			I. History of one or more attacks of rheumatic fever without heart disease	II. History of one or more attacks of rheumatic fever with suspicious rheumatic heart disease†	III. Definite rheumatic heart disease with or without a history of rheumatic fever	Any manifestation of rheumatic fever. Total of I, II and III
A. Control families from general and special pediatric clinics	16	92	0	0	4.3	4.3
B. Control scarlet fever families	19	100	6.0	3.0	3.0	12.0
C. Rheumatic fever families	47	297	7.2	4.6	9.0	20.8
D. Postscarlatinal rheumatism and carditis families	12	58	8.6	6.9	20.3	35.8

* This includes all members of the family who were interviewed and on whom a physical examination was performed, exclusive of that member who represented the basis on which the family was chosen.

† This represents the occurrence of one or more attacks of rheumatic fever in a patient who showed a systolic murmur, which might under other circumstances be interpreted as a functional murmur.

suggested below. The incidence of rheumatic heart disease in the control groups (A and B) was found to be between 3.0 and 4.3 per cent, which is about the same, or a little above, that found by Cohn (16) in his assembled figures obtained by different observers from control families in the Northeastern part of the United States. In the rheumatic families (Group C) the incidence of heart disease also closely approaches that given by Cohn, namely: 8 to 10 per cent, and recalls the observation of St. Lawrence (14), that the familial incidence of rheumatic fever simulates the familial incidence of clinical tuberculosis. In our postscarlatinal rheumatism families (Group D), the highest familial incidence of rheumatic heart disease, and of the total rheumatic manifestations was recorded, the latter being 35.8 per cent.[4]

Although our D Group is a small one on which to draw final conclusions our interpretation of these results is that many examples of postscarlatinal rheumatism or carditis either represent a lighting up of a previously unsuspected, latent or sub-clinical form of rheumatic fever, or represent the manner in which an individual possessed of a " rheumatic diathesis " may react to scarlet fever. Furthermore, if this implication is correct, namely

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[4] A theoretical correction might be applied to this figure (35.8 per cent) representing as it does the per cent of individuals in Group D who at any time had shown evidence of rheumatic fever. Some of these individuals sustained attacks of arthritis or carditis following *Scarlatina sine exanthemate*, in which the scarlatinal nature of the infection was not detected because of the absence of the rash. Such attacks owe their identification to the fact that they occurred coincidently with one or more cases of scarlet fever in other members of the family. Five attacks of this type occurred among members of Group D, two of which occurred in individuals who had suffered from rheumatic fever prior to their attack of *Scarlatina sine exanthemate*. Theoretically all these attacks might be considered as examples of " specific scarlet fever rheumatism or carditis," and consequently should not be listed as examples of rheumatic fever. Their elimination would reduce the incidence figure of the total manifestations of rheumatic fever in Group D from 35.8 to 26.2 per cent—a figure which is still well above that found in the Group C rheumatic fever families.

FIG. 2

A family in which three members had sustained definite attacks of rheumatic fever prior to the occurrence of three cases of scarlet fever in January 1932. All of these cases of scarlet fever were followed by rheumatism. The youngest boy Arthur, who unfortunately had not been examined before, but who gave no previous history of rheumatic fever, developed postscarlatinal rheumatism, associated with suspicious evidences of carditis. When seen 20 months later he had definite mitral insufficiency. The girl Hazel had previously developed aortic and mitral disease in association with an attack of chorea and joint pains at the age of 6. Following her attack of scarlet fever she sustained a severe " reactivation " of her carditis from which she subsequently died in the New Haven Hospital. The mother developed postscarlatinal rheumatism but has not shown evidences of a cardiac lesion. It is interesting to note that the father had also suffered from a severe attack of postscarlatinal rheumatism during adolescence.
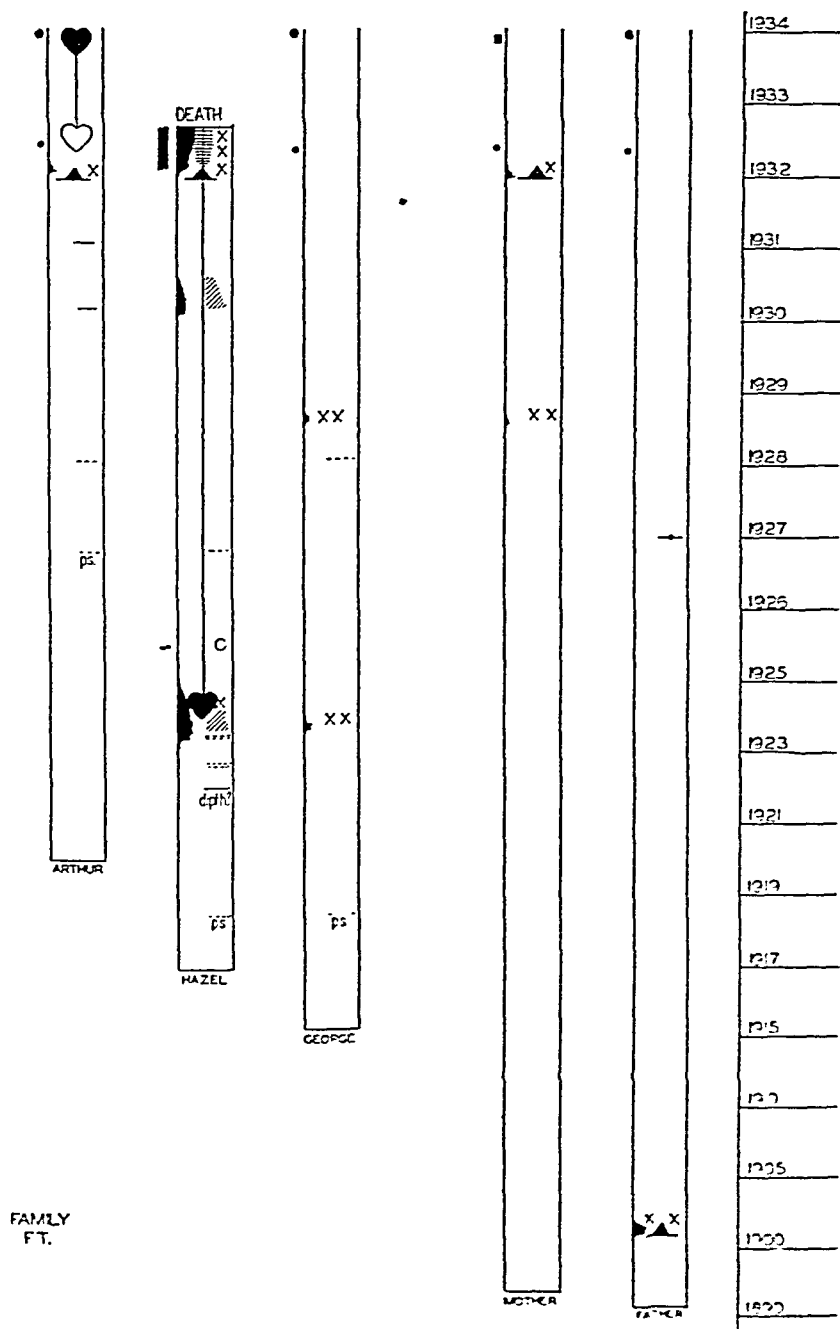
FIG. 2

that the patient who develops postscarlatinal rheumatism comes from a "rheumatic background," it is not surprising that the highest incidence of the manifestations of rheumatic fever should occur in our Group D, for in this group we would have a combination of two factors thought to be of significance in producing the disease rheumatic fever, namely—(i) a rather severe and contagious type of streptococcus infection; and (ii) a "rheumatic background." The presence of the first factor in Group B might also explain why the incidence of rheumatic fever is higher in this group than in the control group A in which both factors are hypothetically absent. The term "rheumatic background" demands some explanation. It has been used to express the idea that the patient comes from a family in which rheumatic fever is known to be present. It may or may not be analogous to the term "rheumatic diathesis," or to Swift's "hyperergic state," (19) or to Coburn's "rheumatic state" (20); all of which represent terms expressive of the major underlying obscure feature of the disease known as rheumatic fever. As the nature of this state is not known, and as it is impossible to measure the relative extent which hereditary or environmental factors play in producing it, the use of a broad or even vague term such as "rheumatic background" to express this idea seems to us at present to be the one of choice for the purpose of the present discussion.

As another means of testing this theory, namely, that if patients who develop these scarlatinal sequelae do so as an expression of this "rheumatic background," it should follow that when multiple cases of scarlet fever occur within families possessing this background, one might expect them to be followed by multiple cases of rheumatism and carditis. That such situations occur is shown by two families taken from Group D, which appear in Figures 2 and 3. Both of these families sustained epidemics of scarlet fever to be followed by multiple cases of rheumatism and carditis, but it will be noted that in both families rheumatic manifestations had been recognized in at least one member prior to the appearance of the epidemic of scarlet fever. It is possible that almost all of the members had previ-

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FIG. 3

This family had recently moved to New Haven and, although first hand records of past illnesses were not available, they had previously been under the care of several physicians, and were quite cognizant of the types of illness which members of the family had sustained. Both the father and mother now have rheumatic heart disease and several of their respective siblings had also had rheumatic fever. No evidences of rheumatic fever were thought to exist in the children prior to the appearance of scarlet fever in this family in February 1931. The three cases of scarlet fever which occurred at this time were followed by rheumatism and carditis in the child Rita; by rheumatism in the child Lawrence, who now presents a systolic murmur at the cardiac apex which may be a "functional" murmur; and by a "reactivation" of rheumatism in the mother. It is possible that the mother may have had heart disease prior to 1931 although she believed that it developed after her attack of scarlet fever.

FIG. 3

ously had mild and unrecognized rheumatic fever. This, as we have already intimated, would seem to be quite likely because, although " primary " cases of postscarlatinal rheumatism are common enough, yet we have observed no instances of this occurring in several members of the same family; or, in our own limited experience, we have never seen a familial epidemic of three or four cases of scarlet fever all complicated by " primary " rheumatism. To illustrate this point further let us take the child Arthur in Figure 2. When viewed individually, his case appears as an example of " primary " postscarlatinal rheumatism followed by postscarlatinal carditis. When viewed in relation to his family, his case might be better classified as rheumatic fever. The same feature holds true for the children Rita and Lawrence in Figure 3.

The frequency with which the situations shown in Figures 2 and 3 occurred, has been roughly determined in Group D, and has been compared with similar information derived from the control scarlet fever families (Group B). This comparison revealed the fact that those members of the Group D families, who sustained an attack of scarlet fever, developed rheumatism or carditis as a complication at a rate which was higher by almost three times than that which occurred after scarlet fever in the families of the Group B controls. It was evident, however, that the high rate of postscarlatinal complications in Group D was due, or at least associated with, the fact that many of the members of these families had sustained frank attacks of rheumatic fever prior to their acquisition of scarlet fever.

DISCUSSION

There are, obviously, obscurities which cloud the results of the comparative study presented in this paper, and not the least of these is the fact that we have compared two poorly-defined clinical entities. For, as the clinical limits of rheumatic fever are ill-defined, so also are the clinical limits of scarlet fever, and there is small wonder that an attempt to define their mutual relationships leads to difficulties. Nevertheless, added to the evidence which exists in medical literature that, in spite of minor differences, the clinical course of postscarlatinal rheumatism and carditis is essentially similar to that of rheumatic fever (11, 12, 13), and that some of the myocardial lesions of postscarlatinal myocarditis are similar to those of rheumatic fever (10, 21, 22, 23), there appears to be still another similarity in so far as the family histories of patients suffering with these conditions are concerned, namely, the familial incidence of rheumatic fever is high in both groups. This last finding has distinct bearing on the crux of the supposed differences between the two conditions, because, as has been already mentioned, if a patient, who has previously had rheumatic fever, sustains an attack of scarlet fever which is followed by rheumatism or carditis or both, these complications are generally regarded as recurrences of rheumatic fever; whereas, if a patient sustains these postscarlatinal com-

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PASSAGE OF NATIVE PROTEINS THROUGH THE NORMAL GASTRO–INTESTINAL WALL [1]

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The view most generally held to-day by the physiologist, chemist and clinician is that undigested antigens are not absorbed through the normal gastro-intestinal wall; and that when such absorption does occur, it is because abnormal or pathological conditions exist, such as stasis, the excessive flooding of the intestines with protein foods, lessened activity of the digestive enzymes, altered conditions of the intestinal mucosa, and greater permeability of the intestinal wall characteristic of new-borns and sucklings. There is some evidence for the absorption of unsplit proteins under normal conditions but this is regarded as a fortuitous occurrence. In the light of the conflicting opinions present in the literature and the important rôle this subject plays in allergy and other conditions as yet not clearly defined, we determined to reopen the problem.

PART I. ANIMAL EXPERIMENTS

Methods

Guinea pigs were obtained from a reliable source. They were observed for a week and we used only those which remained healthy and gained in weight. Both young and mature animals were included in order to determine the influence of age. A large number (493) were studied in order to arrive at some approximation of the incidence of this phenomenon under the conditions to be described.

We used the anaphylaxis test as the biological method of choice to determine whether native antigens had entered the blood stream from the gastro-intestinal tract. As we have previously shown (1), the Schultz-Dale uterine strip method is less reliable as an index of a state of hypersensitiveness. The difficulties in detecting foreign protein in the blood by precipitin methods are considerable, not only because the material is so greatly diluted, but, as has been frequently shown, because it leaves the blood stream so rapidly.

It is essential to use a protein that is foreign to the diet for the reason that animals develop an immunity to foods they eat regularly (2). Such substances as crepitine, originally used by Richet (3) in his experiments on anaphylaxis, or

vegetable proteins such as castor bean or cottonseed, which contain primary toxic substances, were not considered. The protein foods that offered the greatest possibilities for use in our experiments were milk and egg. Egg white was excluded from further consideration because it has been shown to be extremely resistant to digestion. Cow's milk, which is not toxic and is well tolerated by the guinea pig, was used; innumerable experiments already recorded in the literature and large numbers of negative reactions reported in our protocols offer evidence for the absence of any toxic properties in milk when given to the guinea pig intravenously. Fat may tend either to delay the passage of food through the pylorus or to produce diarrhea; we therefore skimmed all cream from the milk. A few control experiments carried out with horse serum are included in the protocols below.

In one series of experiments animals were sensitized orally and shocked by intravenous injection; in another they were sensitized by intraperitoneal injection and shocked orally; and in a final series they were sensitized and shocked orally. Before each feeding experiment the animals were placed in a clean metal cage and observed for 3 to 5 hours, during which period they were not given food. For the oral administration the animals were held gently and the milk was fed by mouth from a glass syringe with a metal tip. Any liquid which dripped out of the mouth was collected in a dish and refed, so that the total amounts ingested were known. This procedure took an inordinate amount of time but we believed it essential to avoid forced feedings.

Measurements were made of the anatomical capacity of the guinea pig stomach and we learned that the stomach of an animal weighing between 250 and 350 grams could hold 15 cc. with ease. Individual feedings were limited to that amount. This was to obviate any criticism relative to excessive flooding of the gastro-intestinal tract.

The animals were kept under normal conditions and observed for 3 to 4 weeks before being retested. Only animals that remained healthy and gained normally during this period were used for the final tests recorded in the protocols.

The respiratory tract serves as an admirable portal of entry (4). Animals which coughed were discarded in order that false results, due to the aspiration of antigen into the respiratory tract, might be avoided.

It must be conceded that experimental procedures can at no time be regarded as being absolutely normal, but certainly tying off loops of the intestinal tract, the use of stomach tubes, the production of stasis, flooding of the intestines and prolonged starvation of the animal constitute distinctly abnormal conditions. Such conditions were strictly avoided in our experiments. We believe that the physical state of the animal, the protein food used, and the care observed in handling and feeding warrant the assumption that our experiments were carried out under generally normal conditions. Particular stress is laid on the fact that the intestinal tract was not traumatized and that the protein food, although foreign to the natural diet of the guinea pig, is non-irritating, non-toxic and well tolerated by the animals.

PROTOCOLS

A. Sensitization of young animals

1. *By feeding repeated doses of milk:* There were 326 animals in this group, 250 to 350 grams in weight, each fed a total of 80 cc. milk. After

an incubation period of 3 weeks an injection of 1 cc. of raw skimmed milk was given intravenously.

One hundred and sixty-one showed symptoms of anaphylactic shock and 165 did not, so that approximately 50 per cent of the animals were sensitized orally. Of the positive animals 51 died in anaphylactic shock (+ + + +), 80 showed marked symptoms with recovery (+ + + and + +) and 30 showed a moderate degree of anaphylaxis with recovery (+).

2. *By a single feeding of milk:* We attempted to learn whether large amounts of antigen are needed to sensitize, or whether small amounts may gain entrance through the normal alimentary wall. There were 22 animals, each given a single feeding of 5 cc. milk. When given an intravenous injection of 1 cc. milk 3 weeks later, 6 showed symptoms of anaphylaxis, 2 of which died in shock and 4 showed symptoms with recovery; 16 were negative.

B. Sensitization of older animals

Can antigens traverse the intestinal wall in older animals as they do in a large percentage of younger animals? In this lot were 47 animals ranging in weight from 600 to 1000 grams.

1. *By feeding milk:* Of this group 19 were fed, as described above, but with somewhat larger doses of milk. On primary injection with 1 cc. milk 8 manifested positive symptoms of anaphylaxis and 11 were negative. Of the 8 positive cases, 3 died in shock and 5 showed definite symptoms of anaphylaxis with recovery.

2. *By feeding horse serum:* In a similar manner 10 mature animals were fed horse serum. After the injection of 1 cc. of horse serum 3 weeks later 1 died in shock, 2 manifested symptoms with recovery, and 7 were negative.

3. *Sensitization of pregnant animals:* There were 18 animals which were fed during the latter part of pregnancy and injected after parturition. Of these 6 were positive, 1 of which died in shock, 5 showed symptoms and recovered, and 12 were negative.

C. Sensitization of new-born through feeding

Many attempts were made to feed animals a few days old, but the mortality was so great that this phase of our work was given up as unfruitful.

Horse serum was used in a few litters. Young animals seem to tolerate this better than milk. We were successful in feeding 1 litter of 4 animals a week old. Proportionately smaller doses were used (see Table I). Of these, 2 died in shock and 1 showed symptoms with recovery following the intravenous shocking injection after an interval of 3 weeks.

TABLE I

Summary of feeding experiments

Number of animals	Type of * animal	Sensitization *		Shock *		Result *	
		Feeding	Ip. inject.	Iv. Inject.	Feeding	Positive	Negative
326	Young	80 cc. M.		1 cc. M.		161	165
19	Old	150 cc. M.		1 cc. M.		8	11
10	Old	60 cc. HS		1 cc. HS		3	7
18	Pregnant	200 cc. HS		1 cc. HS		6	12
4	New-born	20 cc. HS		0.5 cc. HS		3	1
22	Young	5 cc. M.		1 cc. M.		6	16
44	Young	80 cc. M.			5 cc. M.	14	30
8	Old	150 cc. M.			5 cc. M.	1	7
42	Young		5 cc. M.		5 cc. M.	11	31

* Young, 250 to 350 grams; old, 600 to 1000 grams; pregnant, during latter part of pregnancy; new-born, 65 to 75 grams (1 week old); ip., intraperitoneal injection; iv., intravenous injection; positive, definite anaphylaxis with recovery or lethal anaphylaxis; negative, no symptoms or suggestive anaphylaxis; M, skimmed certified milk; HS, normal horse serum; 5 cc. M., 1 small feeding; 80 cc. to 200 cc., several feedings of 15 cc. each.

D. Sensitization by injection; shock by feeding

In this series an attempt was made to learn how rapidly ingested antigens may enter the systemic circulation.

Young animals (250 to 350 grams) were used. They were all sensitized by intraperitoneal injections of 5 cc. of milk, and 3 weeks later were given a feeding of 5 cc. of milk. Animals were observed for about 1 hour after feeding. Each animal was subsequently given an intravenous injection of 1 cc. of milk to determine the degree of hypersensitivity.

There were 42 animals. Oral administration resulted in 11 definite anaphylactic responses, 13 doubtful reactions and 18 negative reactions. Of the group of 11 animals, 1 died in shock, 5 manifested a +++ reaction, 3 a ++ reaction and 2 a + reaction. The reactions occurred from 5 to 30 minutes after the feeding. The subsequent intravenous injection, in the majority of instances, demonstrated that the shock reactions from feeding were generally mild, for 10 animals showing no symptoms from feeding milk died after intravenous injection; nine of the animals which showed doubtful symptoms gave lethal or violent reactions after intravenous injection. However, in 3 instances the shock was quite as severe after

feeding as after subsequent injection.   One animal, indeed, died 3 minutes after feeding.

E. Sensitization by feeding; shock by feeding

1. *Young animals:* In this series there were 44 young animals of which 3 gave a violent reaction, 11 a moderate reaction, 9 a doubtful reaction and 21 a negative response to shock feeding.   In all instances the subsequent intravenous injection of 1 cc. of milk resulted in more intense symptoms of anaphylaxis, indicating that while antigens orally administered may gain entrance into the systemic circulation, in general they do not enter in as large amounts nor as quickly as when given by the intravenous route.

2. *Older animals:* A group of 8 older animals was sensitized and an attempt made to shock them by feeding.   Of these 1 manifested definite anaphylactic shock with recovery, 2 were doubtful, and 5 were negative. All reacted positively after intravenous injection.

These experiments are summarized in Table I.   The large number of animals that failed to give symptoms after the intravenous injection of milk serve as controls with respect to the possible toxicity of the milk.

PART II.   HUMAN DATA

1. *Passage of antigen not common to the diet:* The last 3 years during the course of this study, medical students were given the Prausnitz-Küstner (5) test.   They were injected with the serum of an asthmatic child sensitive to cottonseed and 24 hours later were given a teaspoonful of dried cottonseed by mouth.   These students, in the majority of instances, gave a pronounced reaction at the site of the injection of serum, between 15 and 30 minutes after ingestion.

2. *Passage of antigens common to the diet:* The same method was employed in non-allergic children free from gastro-enteric disturbance.   These children ranged in age from 3 to 12 years.   Eighty per cent of the 20 children tested showed positive reactions to foods common to their diet such as egg, milk and nuts.   We might cite one interesting example.   The serum of an allergic child highly sensitive to lactalbumin was injected into the skin of a 12 year old child, perfectly well and about to be discharged from the hospital.   She abstained from milk throughout the following morning. Within 20 minutes after being given a glassful of milk to drink, she developed a positive local skin reaction, demonstrating the passage of this common food antigen into the blood stream.

Acute attacks of asthma were repeatedly induced in a 9 year old child. sensitive to lactalbumin, by the ingestion of fresh cow's milk in amounts not exceeding several teaspoonfuls.   His serum contained specific antibodies to lactalbumin which we succeeded in transferring by the Prausnitz-Küstner method.   Guinea pigs were also passively sensitized with the serum.   On one occasion, when this patient was tested intracutaneously

with lactalbumin, he developed immediate symptoms of anaphylactic shock from which he recovered.

It is clear from these observations that unsplit antigens, both foreign and common to the diet, can traverse the intestinal wall of normal adults and children. In a highly sensitive allergic individual a small amount of antigen taken by mouth may produce shock.

PART III.   HISTORICAL CRITIQUE

The literature on this subject is most extensive and to present it in its entirety in condensed form would serve only to confuse the reader; we shall therefore only briefly summarize those phases which have the most direct bearing.

Lower animal

In the oldest experiments dealing with this question, the passage of proteins through the intestinal wall was thought to have been demonstrated by finding albumin in the urine after large amounts of protein were fed, particularly egg white (6, 7).

Experiments with loops of intestine were introduced as far back as 1869. Portions of the intestinal tract were exposed and tied off, protein substances injected into the lumen of these loops, and after various intervals the amount of nitrogenous material that remained in the loop was determined. Some (8, 9, 10) who found that a difference existed between the total amount of protein injected and the total nitrogen which remained interpreted this as indicating that undigested protein had passed through the wall of the loop. Such a difference however was not found by all investigators (11, 12). The outstanding experiments performed by this method were done by Mendel and Rockwood (13) who used edestin as the protein substance. The edestin remaining in the loop was recovered, chemically identified, and measured. A reduction was shown. The authors believed that the diminution was due to a passage of some of the edestin in its native state through the wall of the loop.

Mills et al. (14) and Hektoen et al. (15) went further. They fed animals tissue fibrinogen and thyroglobulin respectively, and were able to show the presence of these proteins in the blood stream. Digestion of both these complex protein substances destroys their physiological properties so that their detection in the blood is evidence for their passage in an unchanged state.

However, it was only after immunological methods were introduced that protein specificity could be demonstrated. By the use of specific antisera it was shown that unchanged ingested antigens were present in the blood and urine (16, 17, 18, 19, 20, 21, 22) and conversely, by testing against the ingested antigen, specific antibodies were found in the blood and urine (23, 24, 25, 26, 27). Hamburger is representative of a group of investigators (28, 29, 30) who, though using the precipitin method, apparently found it difficult to demonstrate the presence of antigen in the blood stream after ingestion, and voiced the belief that it occurred only under pathological conditions and particularly in the newborn.

The most convincing evidence for the passage of antigens through the intestinal wall has been brought forth by the use of the anaphylactic method. Animals were directly sensitized and shocked by feeding antigens (2, 3, 22, 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39). The presence of antigen or antibodies in the blood or urine was also determined by indirect anaphylactic tests, i.e., normal

animals were passively sensitized by the blood or urine of animals sensitized through ingestion (37, 40, 41).

It was also shown (42, 43) that the fetus could be actively or passively sensitized to foods ingested by the pregnant female.

Many investigators, as Arloing et al. (44) and Makaroff (45) who followed the teaching of Besredka (46) that proteins did not pass unchanged through the intestinal wall, believed, on the other hand, that if bile or other irritants were fed to the animal before the proteins, the irritation rendered the intestinal mucosa more permeable and permitted the passage of unchanged proteins.

Human data

Czerny and Latschenberger (47) worked with a human being who had a sigmoid fistula. They introduced various protein substances into the fistula and found that there was a diminution in the nitrogen content after a lapse of time. This observation is similar to the results obtained with the intestinal loop experiments performed on animals.

Alimentary albuminuria may occur in the normal individual and is due to the excretion of undigested protein and not to the excretion of endogenous protein resulting from a pathological lesion of the kidney. This has been demonstrated by specific precipitin tests with the excreted protein, and by actively sensitizing normal guinea pigs with the foreign protein present in the urine (7, 16, 21, 26, 48, 49, 50, 51, 52, 53).

The presence of ingested food proteins in the blood stream of normal individuals has been shown by precipitin and indirect anaphylaxis tests (17, 19, 53, 54, 55, 56). Specific antibodies to food proteins found in the blood of allergic individuals have given precipitates with specific antigens, and have passively sensitized normal guinea pigs (41, 57, 58, 59).

Normal individuals, injected intracutaneously with the serum of patients sensitive to some food, have given positive local reactions (Prausnitz-Küstner test) when food to which the patient was sensitive was eaten (60, 61, 62, 63, 64).

Tissue fibrinogen ingested by a group of investigators (14) markedly influenced the blood coagulation time. This complex protein substance must have entered the blood stream unchanged for, as we have already stated, digestion destroys its physiological action.

Innumerable cases of food intolerance have been observed in which positive protein skin tests were obtained, showing that a particular food ingested was the direct cause of the allergic disturbance. This work has been summarized by Rowe (65) and Laroche et al. (66).

Anaphylactic shock and even death are recorded after the ingestion of protein foods (67, 68, 69, 70, 71, 72, 73, 74). Bouteil (75) reported an interesting case of an adult who received 3 rectal injections of horse serum at monthly intervals. After the third injection this patient went into anaphylactic shock.

Ratner (76) has shown that the human fetus can be sensitized in utero as a result of the excessive indulgence in protein foods by non-allergic women during pregnancy. Allergic mothers sensitive to foods may in the same manner transfer specific antibodies to the fetus (77). Thus, the fetus can be actively or passively sensitized to foods in utero and when the infant or child ingests these specific foods for the first time it may manifest allergic reactions.

Certain authors (54, 48, 71, 73, 78, 79) have observed that children suffering from gastro-intestinal disturbances can become sensitized to foods which they tolerated prior to their illness.

It has been reported (80, 81, 82) that individuals who ate horse meat were sensitized to horse serum and manifested anaphylactic symptoms and in some instances have died when given primary injections of horse serum. This was shown to be due to the presence of horse meat antibodies in the blood of these individuals, the result of hippophagy.

PART IV. DISCUSSION

We believe that the historical survey gives sufficient evidence to warrant the conclusion that unsplit antigens enter the blood stream directly from the gastro-intestinal tract. However, we find that little consideration has been given to the important question as to whether this passage occurs through the normal intestinal wall and in the course of normal digestion.

Because they found it difficult to show this phenomenon under normal circumstances with the methods they employed, many investigators introduced artificial and pathological conditions to demonstrate it; and they therefore postulated that only when the permeability of the intestine is increased as a result of intestinal stasis, deficient enzyme action, or damage to the intestinal mucosa, was it possible for unchanged proteins to pass through the intestinal wall. Among other causes accounting for the difficulty in demonstrating the presence of unchanged food antigens and specific food antibodies in the blood stream and urine of normal animals are the following. First must be mentioned the fact that small numbers of animals were used by many of the investigators. Secondly, inadequate amounts of protein have been fed to animals and human beings who subjected themselves to experimentation. Thirdly, the difficulties of detecting foreign proteins and antibodies by the precipitin method are considerable not only because the material is so greatly diluted in the blood and urine, but, as has been frequently shown, ingested foreign proteins absorbed through the intestinal tract leave the blood stream as a rule within a few hours, and disappear from the urine within 24 hours. The attempts made to sensitize animals actively or passively with the blood or urine containing foreign protein or specific antibodies have also met with many failures largely because of the last-named factors.

Pathological lesions and other factors which increase the permeability of the intestinal wall undoubtedly enhance the absorption of proteins, but it is our contention that under normal conditions the absorption of proteins occurs with greater regularity than is generally believed.

As we have already pointed out experimental procedures can at no time be regarded as being carried out under absolutely normal conditions. Our experimental conditions were normal in so far as the gastro-intestinal tract was not traumatized, and in so far as we used no chemical irritants. We chose the direct anaphylaxis method as that subject to fewest criticisms. Cow's milk, while it is not natural to the diet of the guinea pig, contains no primary toxic substance. Our animals were observed for three weeks after the original oral administration of the foreign protein and during this period

they showed no evidence of diarrhea and no loss in weight; at the end of this time, before they were retested, they appeared healthy and active. We assumed therefore that the experiments were carried out under generally normal conditions.

Our experiments on a large number of animals show that at least 50 per cent can be sensitized through natural ingestion and that the passage of these antigens takes place in the adult as well as in the newly-born animal. Antigens, when fed, generally enter the circulation in small amounts. Though a single small dose may occasionally sensitize, moderately large amounts are necessary to sensitize animals with any degree of regularity. At times, also, antigens are absorbed in sensitized animals in large enough amounts to produce shock reactions which are as profound as those observed after intravenous injection.

In the human subject our observations show that cottonseed as well as foods common to the diet, such as milk, when taken into the stomach may enter the circulation in demonstrable amounts. This occurs not only with allergic patients but also with normal individuals and in the adult as well as in the young.

Schloss and his co-workers (56), using immunological methods (precipitin and indirect anaphylaxis tests), showed that ingested native proteins (milk, egg, almond) enter the blood in normal young children. Walzer et al. (62), who applied the Prausnitz-Küstner test, found that 88 per cent of normal adults whom they studied showed the presence of proteins of egg and fish in the blood after ingestion. Walzer particularly stresses the physiological nature of this passage and, because of its frequent occurrence in the average individual, believes it to be a normal phenomenon.

Proteins may enter the blood stream from any part of the bowel even as low down as the rectum. The entrance of these antigens into the circulation apparently takes place below the stomach for it is well established (83) that there is little absorption of any kind from the gastric mucous membrane. It is doubtful whether any chemical stimulus of the gastric mucosa is needed to open the pylorus for it relaxes at intervals and if the gastric contents are under any pressure the liquid part is squirted out while the more solid parts are retained. The intestinal contents are propelled by large rapid waves, which run down the bowel from time to time, called "peristaltic rush" by Meltzer and Auer (84). It is conceivable therefore that antigens in fluid form can pass the pylorus and may enter the small and large intestines in an exceedingly short time. This would explain the death that occurred in one of our animals within 3 minutes after ingestion of the foreign protein; the rapid passage of cottonseed and milk in the human subjects after ingestion, and the observations of Mills et al. (14) that ingested fibrinogen entered the circulation within $2\frac{1}{2}$ minutes.

Another factor which may assist in the absorption of unsplit proteins is to be found in the fact that all proteins are not digested with equal

avidity. Many vegetable proteins escape digestion. It has been shown by Mendel and Lewis (85) in the human being, and Bateman (86) in the animal, that raw egg white largely escapes digestion. It has also been demonstrated that the proteins of raw milk are not readily digested and therefore the soluble whey proteins might easily be absorbed.

Thus, the inaccessibility of proteins to enzyme action and their rapid passage through the intestinal tract might allow for the ready entrance of undigested proteins into the blood stream. The process must be in the nature of a purely mechanical filtration because of the rapidity with which the proteins enter the blood stream.

If antigens enter the circulation regularly, one must attempt to explain why all individuals are not sensitized to these foreign proteins. Quantity and time are largely essential in the mechanism of sensitization. If antigens enter in small amounts and at frequent intervals, it has been shown by Wells (2), Schloss and his co-workers (56), and Laroche et al. (66) that an immunity is established. On the other hand, if antigens enter in particularly large amounts and at infrequent intervals, then the animal or man may become profoundly sensitized and the subsequent ingestion of even small amounts of antigen may produce allergic manifestations, shock, and even death.

It is feasible to speculate that throughout the ages animal and man must have developed defense mechanisms, for otherwise they would have succumbed to the ingestion of new and strange protein foods. The first line of defense is the hydrolysis of the ingested proteins by the digestive enzymes; next, the general impermeability of the intestines to proteins in a native state. The second line of defense after the antigen has entered the blood stream consists in the development of specific antibodies which may divert the absorbed protein from the sensitized cells, thereby rendering it harmless; and next, the important function of urinary excretion which tends to rid the circulation of foreign proteins. A third line of defense which does not pertain to body processes is the development of various methods of cooking and preparing foods which render proteins more digestible; and lastly, the general tendency of man and animal to indulge in new foods sparingly and to adhere to a more or less limited diet.

We believe that although abnormal conditions, which tend to increase intestinal permeability facilitate the passage of proteins through the intestinal wall, absorption of protein occurs under normal conditions with greater regularity than is generally held. This normal absorption may conceivably serve the useful purpose of constantly maintaining the human body in a state of immunization against habitual protein diets.

CONCLUSIONS

Mature, as well as young animals can be sensitized and shocked by the oral administration of protein foods. The incidence in a large series of

animals w shown to be as high as 50 per cent. The experiments were carried o within physiological limits. We believe that these experiments offer con sive evidence for the fact that unsplit proteins pass the intact intestinal all under normal conditions.

In the uman being it is shown that proteins, natural as well as foreign to the die pass the intestinal wall and may enter the blood stream under physiolog al conditions at all ages.

The b ly is provided with certain defensive measures which impede the entrance proteins in an unchanged state into the blood stream and tissues. These in ide the general impermeability of the intestines, the digestive enzymes, pecific antibodies and the excretory function of the kidneys. Denatura on of proteins by culinary processes also aids the body in this defense. When the defense mechanisms fail to act and native antigens enter the blood stream in large enough amounts, man or animal may become pro undly sensitized and the ingestion of small amounts of antigen thereafte will produce allergic manifestations and may even result in death.

Altho h abnormal or pathological conditions of the intestinal mucosa increase testinal permeability and facilitate the passage of unchanged proteins, we elieve that under normal conditions absorption of unaltered protein occu with great regularity. This normal absorption may conceivably serve the seful purpose of constantly maintaining the body in a state of immuniz ion against habitual protein diets.

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STUDIES IN CONGESTIVE HEART FAILURE

XXII. A METHOD FOR OBTAINING "MIXED" VENOUS BLOOD BY ARTERIAL PUNCTURE

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INTRODUCTION

Burwell and Robinson (1) devised a method for determining the cardiac output of man. These authors did not apply their method to patients with cardiac disease, for it was believed that in such cases diffusion of oxygen through the pulmonary membrane might be impaired, and hence the tension of oxygen in the rebreathing bag might be different from that of the venous blood. In an endeavor to overcome this possible cause of error we have developed a method in which "mixed venous blood" is taken from a peripheral artery while the subject breathes "venous air" which has been obtained by previous rebreathings. The technical details of our method are similar to those of the Burwell and Robinson procedure except for the final rebreathing, during which the blood samples are drawn. In the following pages data are presented which seem to indicate that the method allows one to obtain, from an artery, blood which has passed unchanged through the lungs.

PRELIMINARY ADJUSTMENT OF THE AIR IN THE LUNGS

We found, as did Burwell and Robinson, that one can best obtain an accurate equilibrium by washing out the lungs with a low-oxygen high-carbon dioxide gas mixture, just before the subject begins to rebreathe from the bag. Unless this is done the bag is diluted at the beginning of each rebreathing with residual air which is so much richer in oxygen than the venous air that a true equilibrium cannot be obtained. In order to remove the excess oxygen from the residual air as rapidly as possible we prepared in a Tissot spirometer 60 to 80 liters of a gas mixture containing approximately 92 per cent nitrogen, 6.5 to 7.0 per cent carbon dioxide, and 0.5 to 1.5 per cent oxygen. Following a forced expiration the tap was turned so as to connect the subject to the valve and he then rapidly took two to four breaths from the spirometer, the expired air being discarded through the valve. In order to determine the optimal amount of " washing " necessary to bring the gas tensions in the residual air to approximately

the venous level numerous analyses were made of alveolar air following varying amounts of "washing." The data are plotted in Figure 1.

Alveolar oxygen (black symbols) and carbon dioxide (hollow symbols) are plotted against the volume of the air breathed in the preliminary adjustment of the lung air before rebreathing from the bag was begun. The
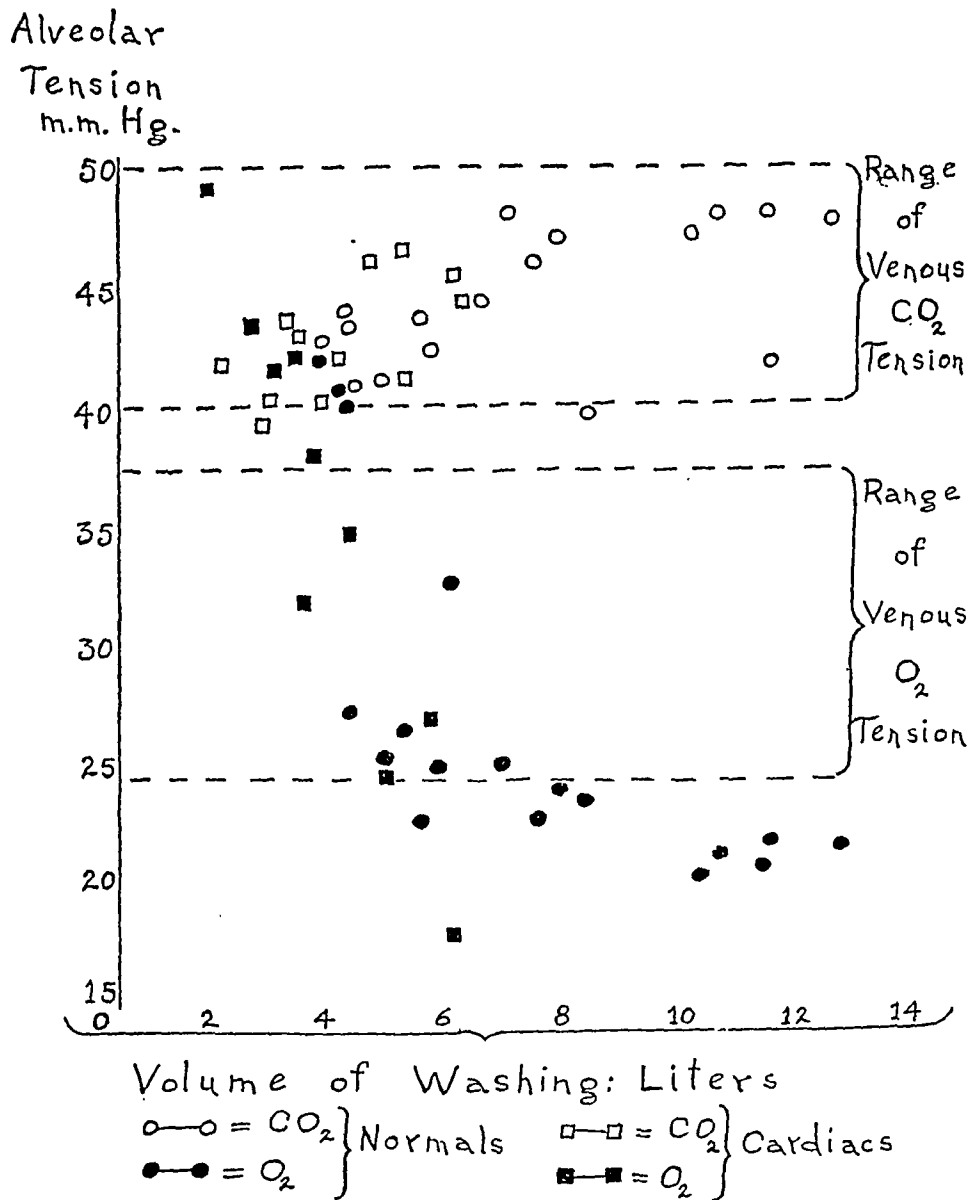
FIG. 1. PRELIMINARY ADJUSTMENT OF THE AIR IN THE LUNGS

gas mixture used in the adjustment contained about seven per cent carbon dioxide and one per cent oxygen. Almost all of the values for carbon dioxide fall within the range of the normal venous carbon dioxide tension. However, the oxygen values for both normal subjects (circles) and persons with cardiac disease (squares) tend to be above the venous oxygen

tension when the volume of washing is less than three liters and below this range when the volume of washing is greater than seven liters. Values within the desired range are best obtained when the volume of air breathed is five to six liters, when the gas mixture has the composition described. This can be breathed in two breaths by normal subjects, whereas persons with cardiac disease require three to four breaths depending on the degree in which their vital capacities are reduced. In order to be certain that the volume of " washing " was approximately correct the spirometer scale was marked before each rebreathing, and the tap was turned into the rebreathing bag as soon as the subject had " washed " his lungs with the desired amount of gas. With well trained subjects the duration of this phase of the procedure was three to six seconds, leaving approximately twenty seconds for equilibration between the blood and the air in the bag.

THE ATTAINMENT OF EQUILIBRIUM BETWEEN THE VENOUS BLOOD AND THE GAS IN THE REBREATHING BAG

The oxygen and carbon dioxide tensions of the " mixed " venous blood of normal persons are in the general region of thirty and forty-five millimeters of mercury, respectively. Consequently, we have prepared the rebreathing bag with about nine liters of a gas mixture of 4 to 4.5 per cent oxygen and 6.5 to 7 per cent carbon dioxide. In a large series of observations in which analyses of the contents of the bag were made after repeated rebreathings we found, as Burwell and Robinson did, that subjects with normal lungs will usually reach and maintain reasonably constant values, i.e., within two millimeters—for oxygen and carbon dioxide within six to eight rebreathings. Ten to twelve repetitions may be necessary in persons with mild cardiac failure. However, the fact that gas tensions become constant does not necessarily indicate that they are in equilibrium with the venous blood—for at the beginning of each rebreathing the air in the bag is diluted with the residual pulmonary air containing a gas mixture which, even though the " washing " has been correctly done, may be several millimeters different from the venous tension. Hence, if the volume of air used in washing the lungs and the duration of the rebreathing are constant one might obtain the same gas mixture in the bag at the end of each rebreathing, even though this mixture was not in equilibrium with the venous blood. In order to test this possible source of error a group of observations was made in which the subject rebreathed alternately from two bags of different oxygen content, one of them having less and the other more oxygen than the amount corresponding to the tension of the venous blood. It was found that in normal subjects and in patients with mild congestive failure the two bags reached the same oxygen content after repeated rebreathings. An example of such an experiment is shown in the broken lines of Figure 2. On the other hand, when the same procedure was repeated in cases with severe congestive failure, the two bags did not always

reach the same oxygen content. Such a failure to obtain equilibrium occurred most commonly in persons with syphilitic aortic insufficiency. It indicates that the method is inapplicable to such cases.

During the final rebreathing it is necessary to exceed somewhat the minimum circulation time in order to obtain blood samples. The return of
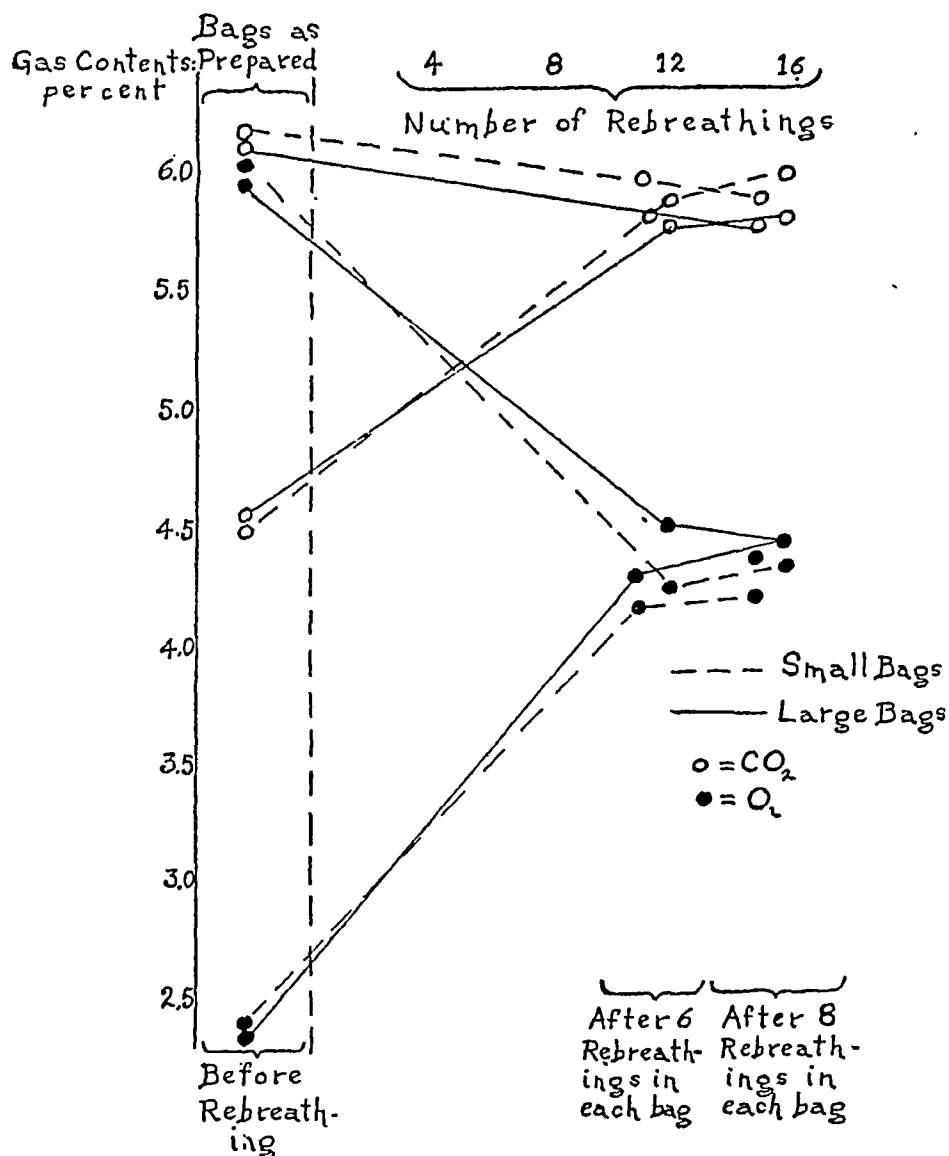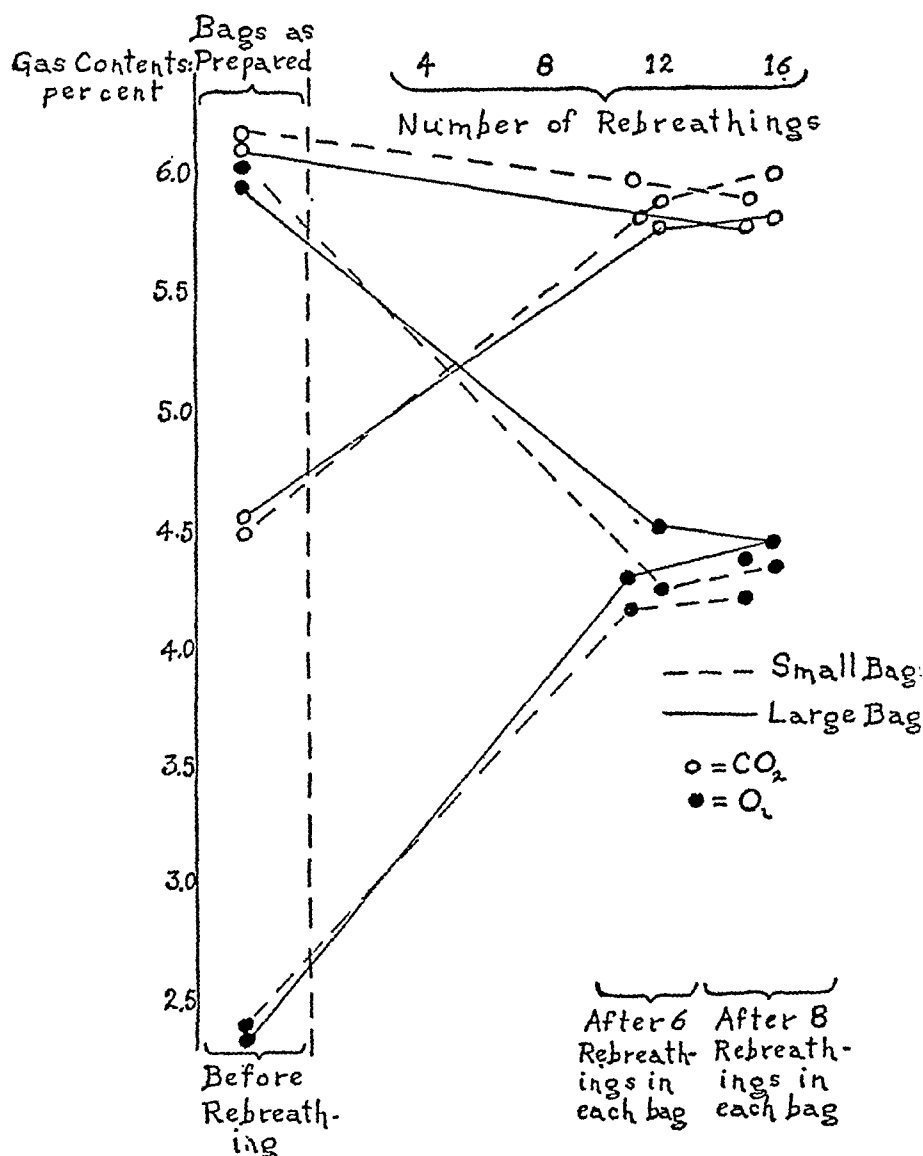
FIG. 2. THE ATTAINMENT OF EQUILIBRIUM BETWEEN THE VENOUS BLOOD AND THE GAS IN THE REBREATHING BAG

coronary or other blood will tend to lower the oxygen concentration in the bag, but this error will be less the greater the gas volume in the rebreathing system. In order to determine whether the volume of gas used makes any appreciable difference in the final tensions arrived at, experiments have been done in which each rebreathing period was divided into two parts, two

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The observations charted in Figure 4 were similar to those portrayed in the previous figure but the subjects used were patients with mild cardiac failure. The results show that when relatively large gas volumes, i.e., nine liters, are used in the bag, recirculation does not usually cause significant changes in the gas contents until rebreathing is prolonged beyond forty-five

FIG. 4. INFLUENCE OF THE DURATION OF REBREATHING IN PATIENTS WITH MILD CARDIAC FAILURE

seconds. The data in Figures 3 and 4 indicate that both in normal subjects and in persons with mild cardiac failure, significant changes in the composition of the lung-bag air do not usually occur before 40 seconds when large gas volumes—nine liters—are used in the bag. After forty-five seconds recirculation causes sufficient change in the gas tensions to render the method inaccurate. Hence, the final rebreathing, during which blood

The object of the experiments charted in Figure 3 was to determine the maximum duration of rebreathing before recirculation caused significant changes in the gas content of a rebreathing bag containing nine liters of " venous air." Repeated rebreathings, which did not last longer than twenty-five seconds, were done until constant values for oxygen and carbon
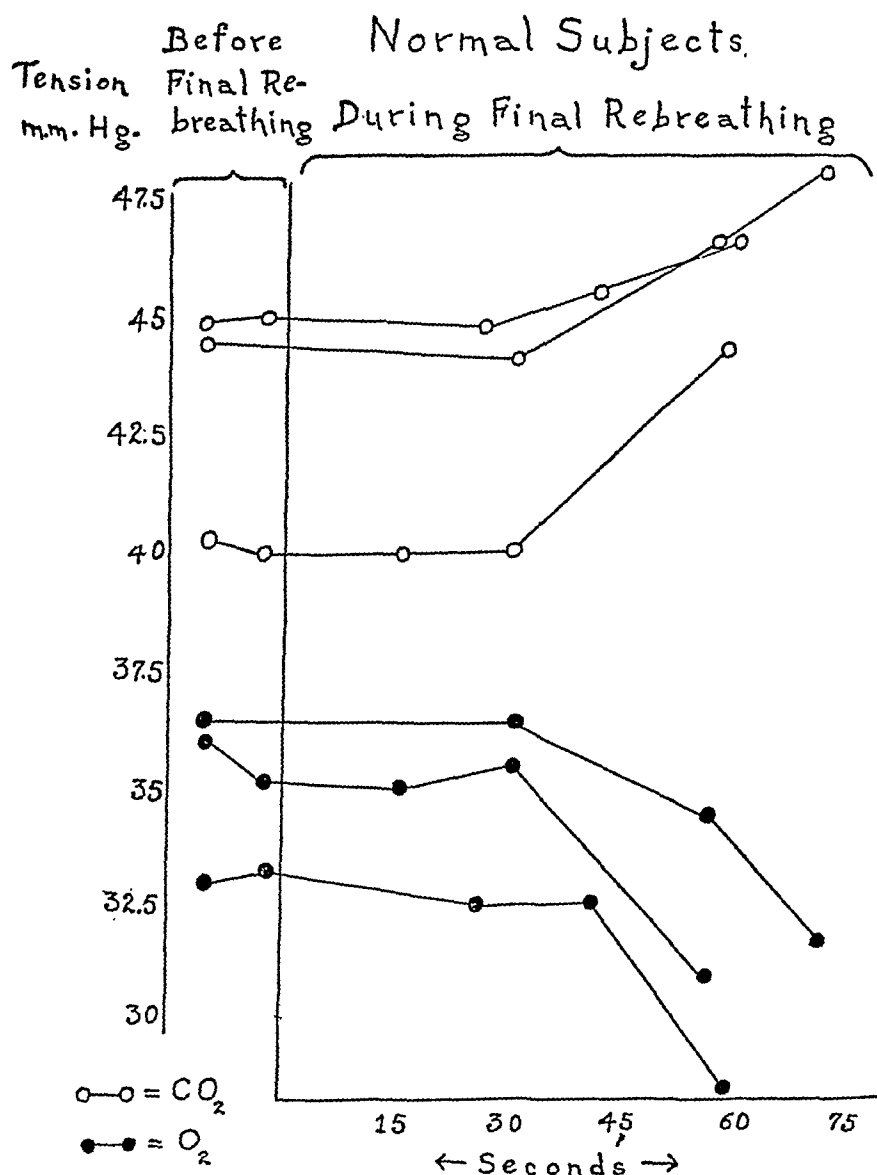
FIG. 3. INFLUENCE OF THE DURATION OF REBREATHING IN NORMAL SUBJECTS

dioxide were obtained in the bag.   The effect of a prolonged final period of rebreathing was then observed.   During and at the end of it gas samples were taken into evacuated sampling tubes.   The observations indicate that in normal subjects recirculation does not cause significant changes in the oxygen and carbon dioxide contents until rebreathing is prolonged more than forty seconds.

The observations charted in Figure 4 were similar to those portrayed in the previous figure but the subjects used were patients with mild cardiac failure.   The results show that when relatively large gas volumes, i.e., nine liters, are used in the bag, recirculation does not usually cause significant changes in the gas contents until rebreathing is prolonged beyond forty-five

FIG. 4. INFLUENCE OF THE DURATION OF REBREATHING IN PATIENTS
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samples are obtained, should not be longer than forty seconds in normal subjects or forty-five seconds in persons with cardiac failure. In all instances gas samples should be taken from the bag during and at the end of the final rebreathing. If the gas tensions in these samples do not agree, within one or two millimeters, with those obtained from the bag earlier in the rebreathing the entire experiment should be discarded.

CURVES OF THE ARTERIAL OXYGEN DURING THE REBREATHING OF "VENOUS AIR"

After the preliminary rebreathings had been completed, arterial blood was obtained from the femoral artery while the subject breathed air. With the needle still in the vessel the lungs were "washed" and the final rebreathing was begun. The arterial blood, which maintained its bright crimson color for the first ten or fifteen seconds, became blue after about twenty seconds. As soon as the change in color was well marked sampling was begun and specimens were taken as rapidly as possible during the succeeding thirty seconds. When the oxygen content of these samples was determined, curves of the type illustrated in Figure 5 were obtained. After an initial lag, which is not shown in the curves, the oxygen diminished rapidly and then became constant for a time. This plateau usually occurred between the twenty-fifth and forty-fifth seconds after the subject began to breathe the low-oxygen mixture and was followed by a further decrease in the oxygen content of the blood.

Failure to obtain a plateau in the oxygen curve occurred in a number of instances and was usually due either to difficulty in getting the blood samples or to some error in the respiratory manipulations. Such experiments have been discarded. When a plateau was found it has been assumed, however, to represent the oxygen content of the mixed venous blood. This assumption has been based on the following considerations:

1. On theoretical grounds it appears likely that the lag in the decline of the blood oxygen is dependent on the time required for previously arterialized blood to pass from the lungs to the periphery. The initial decrease in the oxygen is probably due to a mixture of such blood with other blood which has been equilibrated with venous air. When all of the blood which had been exposed to air before the beginning of rebreathing has passed out of the arterial system the curve becomes flat until recirculation produces sufficient reduction in the oxygen content of the bag to cause a secondary decrease in the blood oxygen. The plateau in the curve probably represents venous blood which passes unchanged through the lungs.

2. Experiments were performed on dogs as follows: The animals were anesthetized with barbital and placed in a small Drinker respiratory apparatus, with a detachable top. The respiratory procedure was then carried out in the same way as in our observations on man, the lungs being "washed" with the nitrogen-carbon dioxide mixture and then several

breaths being taken to and from a rebreathing bag containing the gas mixture as used in observations on man.   With suitable adjustment of the apparatus it was possible to produce large respiratory excursions.   Because of the shorter circulation time of dogs the procedure was not prolonged more than fourteen or fifteen seconds.   Following twelve or more repeated
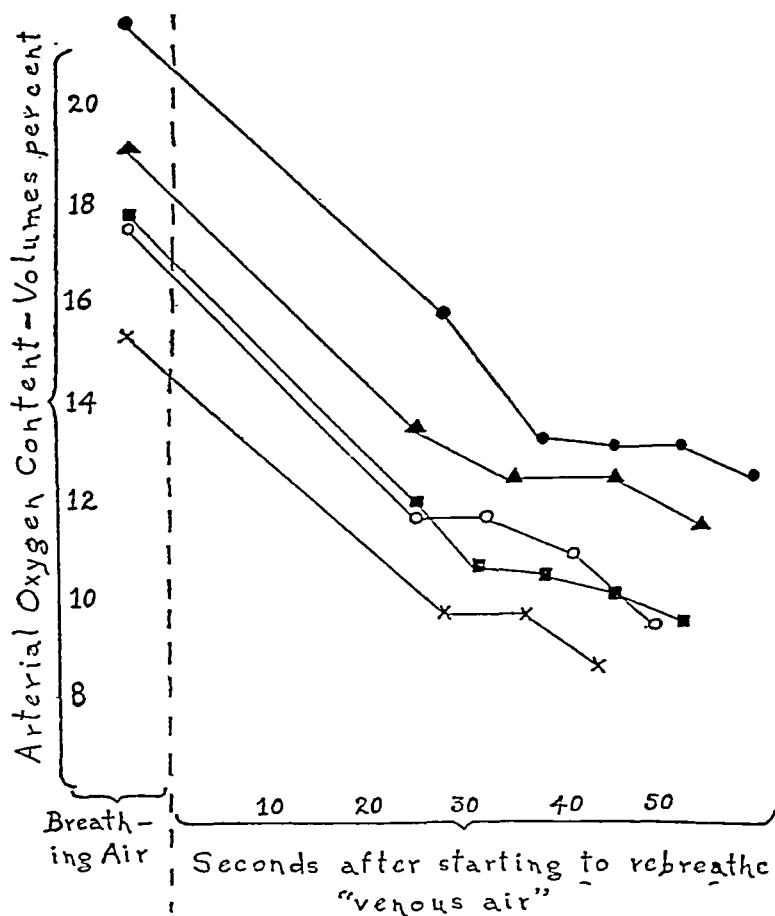
FIG. 5.   THE OXYGEN CONTENT OF ARTERIAL BLOOD BEFORE AND DURING
THE REBREATHING OF " VENOUS AIR "

The plateau in the curve is believed, for reasons mentioned in the text, to represent the oxygen content of mixed venous blood.

rebreathings gas samples were taken from the bag, in order to determine whether the oxygen and carbon dioxide tensions had become constant. The top of the apparatus was then removed and blood was drawn from the right ventricle by puncture of the chest wall.   The top was then replaced and a final rebreathing was done, during which repeated blood samples were drawn from a cannula in the carotid artery.

Examples of six such experiments are shown in Figure 6.   In three of them no plateau occurred in the blood oxygen curve, but in the other three instances a plateau was found and in each of them it corresponded accurately with the oxygen content of the blood obtained from the right ventricle.   These observations appear to justify the assumptions that a similar relationship exists in man and that the procedure described enables one to obtain mixed venous blood from a peripheral artery.
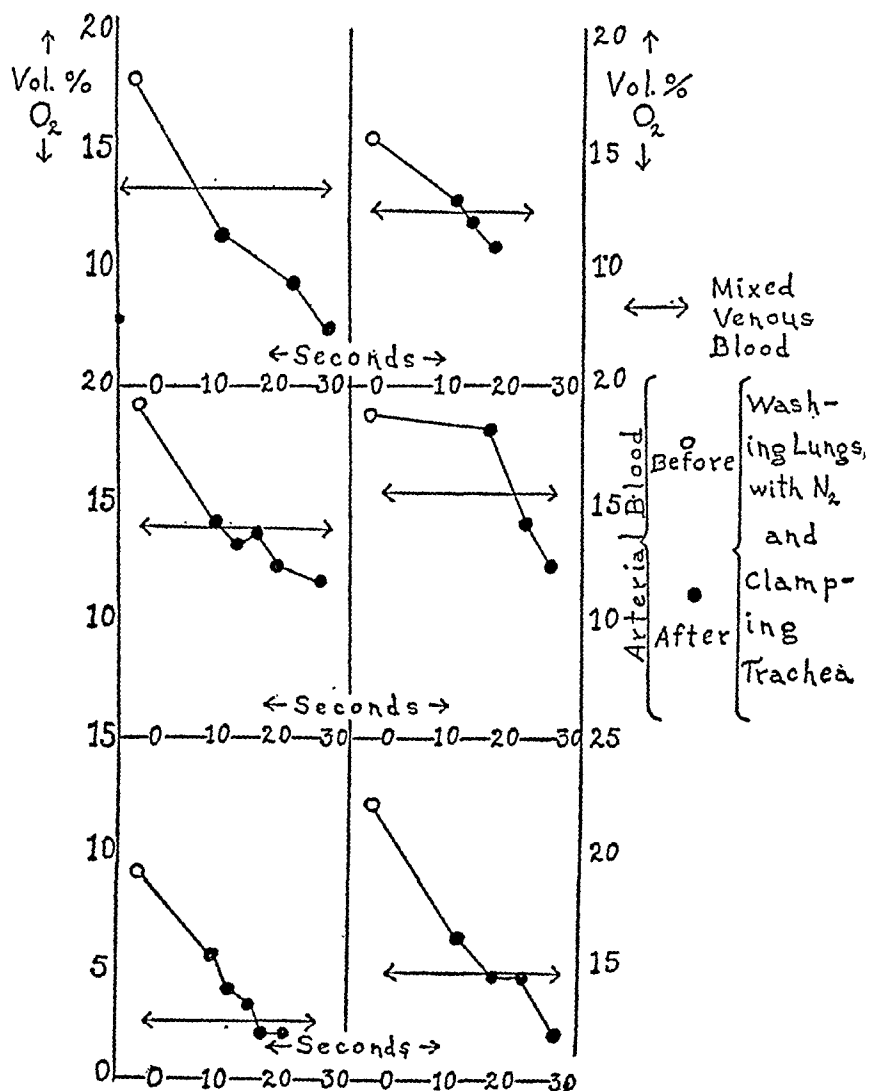
FIG. 6. "VENOUS AIR" OBTAINED IN DOGS BY REPEATED REBREATHINGS

In three animals a plateau was found and in each instance it corresponded accurately with the oxygen content of the mixed venous blood drawn directly from the heart by puncture of the chest wall.

APPLICATION OF THE METHOD TO PERSONS WITH CARDIAC DISEASE

The respiratory procedure causes well marked dyspnea in normal persons and even greater distress in patients with cardiac disease.   It is there-

fore inapplicable in persons with advanced congestive failure. Neurotic subjects are also unsuitable. The rather severe arterial anoxemia might precipitate an attack of angina pectoris in persons with either coronary arteriosclerosis or syphilitic aortic insufficiency. In such cases the procedure should not be applied.

Experiments have been made in an attempt to determine whether edema of the lung bases renders the method inaccurate. Two hundred cubic centimeters of fluid were introduced into the lungs of dogs *via* the trachea. The respiratory procedure was then carried out as described above. In Table I

TABLE I

A comparison in dogs of the venous plateau and direct Fick methods for determining the cardiac output

Animal number	Conditions	Arterial oxygen saturation	Cardiac output			
			By oxygen		By carbon dioxide	
			Direct Fick method	Venous plateau method	Direct Fick method	Venous plateau method
		per cent	*liters*	*liters*	*liters*	*liters*
D_1	Normal		1.85	2.06	2.09	2.44
D_2	Normal	93.8	1.81	2.00	1.94	1.88
D_3	Normal	92.4	1.21	1.04	1.19	1.18
D_4	100 cc. saline solution in lungs	74.8	1.41	1.40	1.58	1.52
D_5	100 cc. saline solution in lungs	82.8	2.00	2.06	2.05	2.05
D_6	200 cc. saline solution in lungs	77.4	2.18	No plateau	2.50	2.31

are shown the results of such experiments in which the cardiac output was determined by the direct Fick method and by the venous plateau method. When a plateau was obtained in the blood gas curves, it corresponded satisfactorily with the gas contents of the blood obtained from the right ventricle. The experiments indicate that " edema " of the lungs of sufficient severity to reduce the arterial oxygen saturation to eighty per cent or less does not invalidate the method.

A more detailed consideration of the applicability of the method in persons with cardiac disease has been given in another study of this series (2).

THE DETERMINATION OF THE CARBON DIOXIDE CONTENT OF THE MIXED VENOUS BLOOD

Thus far our discussion has been concerned chiefly with the venous oxygen. The same procedure allows one to determine the carbon dioxide

described in another study of this series by Grollman, et al. (2). The latter procedure which involves a modification of the original acetylene method causes practically no discomfort to the subject and does not necessitate arterial puncture. When the two methods are properly applied they yield similar results. The venous plateau method has been described in detail in the present paper because we believe that its agreement with the modified acetylene procedure furnished additional evidence for the validity of the latter, which is based on an entirely different principle. In the study of the cardiac output in cardiac disease such safeguards are necessary. Their neglect has already led to questionable conclusions based on methods of doubtful validity.

SUMMARY

A modification of the method of Burwell and Robinson for determining the gas contents of " mixed " venous blood has been described. The procedure depends on obtaining blood from a peripheral artery while the subject breathes a gas mixture which has been equilibrated with his venous blood by previous repeated rebreathings.

The several procedures involved in the method have been checked by various experiments.

Application of the method to dogs has demonstrated that the values found for the blood gases by this indirect method are in close agreement with the gas contents of blood obtained by puncture of the right ventricle. The presence in the lungs of sufficient fluid to produce well marked arterial anoxemia does not invalidate the results.

The method is difficult to employ and involves considerable discomfort to the subject. Its agreement with the modified acetylene procedure constitutes additional evidence as to the validity of the latter in subjects with cardiac disease.

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PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION. IV. BLOOD ELECTRO-LYTE AND HEMOGLOBIN CONCENTRATIONS [1]

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In previous papers of this series (1) the authors have shown that de-rangements of the functions of the liver and kidneys play important rôles in the physiological disturbances following the intravenous injection of diphtheria toxin in rabbits. The following paper reports the results of the investigation of the changes in concentration of serum electrolytes and hemoglobin.

PROCEDURE

Rabbits weighing approximately two kilograms were used as the experi-mental animals. They were maintained on a fasting regime, which began 24 hours before the injection of the toxin and continued thereafter. Water was always present in the cages. Three groups were studied. The first group of 5 animals served as fasting controls. Each of the second group (6 animals) re-ceived intravenously 3 minimal lethal doses of diphtheria toxin. The third group (8 animals) each received 0.8 intravenously. (Previous work had dem-onstrated that these quantities of toxin would cause death in from 2 to 3 days and 4 to 7 days, respectively.) The blood electrolyte studies were made 1 or 2 days before the expected death of the animals. Because of the relatively large amount of blood needed (20 to 25 cc.), cardiac punctures were employed. The blood was collected under oil, allowed to clot, centrifuged, and the serum re-moved as soon as possible. Before withdrawal of the blood, the animal was anesthetized by the intraperitoneal injection of 0.5 cc. of 10 per cent sodium amytal solution per kilogram of body weight. This procedure was used in order to eliminate the effects of struggling. The hemoglobin determinations were made before the injection of the toxin, and every day thereafter. The blood was obtained from a freely flowing wound made by a small incision through a marginal ear vein.

The methods employed for the chemical determinations were the following: bicarbonate, manometric method of Van Slyke and Neill using 0.2 cc. (2); chloride, Patterson micromodification of the open Carius method (3); phos-phate, Benedict and Theis (4); total base, Stadie and Ross modification of the Fiske method (5) without removal of phosphate; sodium, Barber and Kolthoff method (6); potassium, Shohl and Bennett method (7); nitrogen, Kjeldahl method, using 1 cc. (the factor 6.25 was used to obtain per cent protein); non-

nonprotein nitrogen values.   For this reason, it is felt that the elevation in inorganic phosphate parallels the degree of diphtheritic intoxication.

Table I gives the results of analyses of gastric contents.   The total contents were recovered, weighed, and filtered through gauze.   The acidity was titrated with phenolphthalein as indicator, and the total base and chloride were determined by the methods used in the analyses of serum.   The re-

TABLE I

Chloride and total base of gastric contents in normal and diphtheritic rabbits

Rabbit number	Toxin	Blood nonprotein nitrogen	Weight	Gastric contents			
				Concentration		Total amounts	
				Cl	Total base	Cl	Total base
	minimal lethal dose	*mgm. per cent*	*grams*	*m. Eq. per liter*	*m. Eq. per liter*	*m. Eq.*	*m. Eq.*
1	0		82	106	19	8.6	1.6
2	0		116	190	26	22.0	3.0
3	0		52	116	26	6.0	1.4
4	0		100	174	25	17.0	2.5
AVERAGE				146 ± 36	24 ± 2	13.4 ± 6.1	2.1 ± 0.6
5	0.8	340	78	180	49	14.0	3.8 .
6	0.8	180	103	133	32	14.0	3.3
7	0.8	75	56	143	36	8.0	2.0
8	0.8	210	42	152	31	6.3	1.3
9	0.8	270	65	147	42	9.6	2.7
AVERAGE				151 ± 12	38 ± 6	10.4 ± 3.5	2.6 ± 0.8
10	3.0	220	154	106	25	16.0	3.8
11	3.0	240	151	153	51	23.0	7.6
12	3.0	235	143	147	29	21.0	4.2
13	3.0	259	66	129	35	8.5	2.3
14	3.0	214	106	128	34	14.0	3.6
AVERAGE				133 ± 14	35 ± 7	15.9 ± 4.3	4.3 ± 1.3

sults are expressed both in terms of concentration and total amount.   Since the dried weight, including the food present in the stomach, only constituted about ten per cent of the total weight, the results were calculated in terms of total weight of gastric contents.   This did not introduce an error significant for our purposes.   The table demonstrates in the three groups no significant difference in either concentration or total amount of electrolytes in the gastric juice.

In Chart II are graphically presented the daily hemoglobin values in two normal and four diphtheritic rabbits, two of which were injected with the larger dose and two, with the smaller dose of toxin. A total of 4 normal and 10 diphtheritic animals were studied in this manner. The results agree sufficiently so that only representative experiments need be cited. One may note a progressive hemoconcentration, as evidenced by the in-
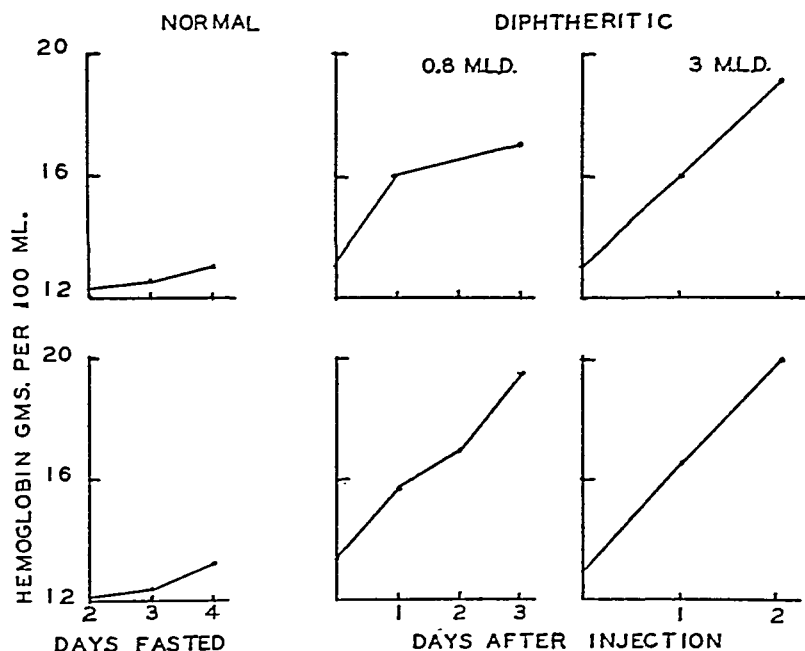
CHART II

THE CONCENTRATION OF HEMOGLOBIN IN NORMAL AND
DIPHTHERITIC RABBITS

crease in hemoglobin values during the course of the intoxication. Unfortunately, accurate measurements of the fluid intake were not made. However, no apparent difference in this respect between the control and experimental animals was noted except that the fluid intake of the diphtheritic rabbits was low during the last 24 hours. Since the increase in concentration of hemoglobin is 30 to 45 per cent of the initial concentration, the lack of change in serum protein indicates a decrease in the total amount of circulating serum protein.

DISCUSSION

While the changes in concentration of serum electrolyte and hemoglobin found in rabbits following intravenous injection of diphtheria toxin cannot

be assumed to represent exactly the condition of the blood in diphtheria, analogous disturbances have been reported in patients. Martinson et al. (10) found reduction in whole blood chloride, whole blood total base and serum bicarbonate. While the electrolyte determinations on whole blood cannot be interpreted with certainty without knowledge of the cell volume, the results are suggestive.

Martinson et al. (10) and Wladimirowa (11) found organic acid in the blood of diphtheritic patients increased as compared to that of normal individuals. These results were obtained by titration with the application of a correction for creatin. While this method is subject to errors it probably represents approximately the change in organic acids. Csapó (12) found an increase in urinary organic acids.

Harding (13) and Hottinger (14) called attention to the high hemoglobin concentration and high erythrocyte count in malignant diphtheria.

Low concentration of inorganic phosphate was found in malignant diphtheria by Lesné, Zizine and Briskas (15). If this finding should be confirmed in severe cases with elevation of nonprotein nitrogen, it constitutes a definite difference from the reactions in rabbits injected with toxin. Feigl (16) found 16, 20 and 24 mgm. per cent of inorganic phosphorus in 3 cases of acute yellow atrophy of the liver. Since pathological and chemical evidences of liver injury are so marked in diphtheritic intoxication, elevation of phosphate may depend on hepatic injury. However, since high serum phosphate frequently occurs in renal disease accompanied by azotemia, the hyperphosphatemia in diphtheritic intoxication may be referable to the renal lesions of the disease.

The changes in rabbits are closely analogous to those found in shock, where diminution of blood volume is accompanied by decrease in the concentration of serum electrolytes. Low concentration of serum electrolytes is also characteristic of febrile states and a number of toxic conditions (17). The changes are apparently not principally dependent on an inadequate supply of salts but represent an unexplained disturbance in the usual concentration of the electrolytes. Since nephritis is frequently accompanied by low serum electrolytes, the renal lesions in the diphtheritic rabbits might produce similar disturbances. The studies of gastric contents demonstrate that the diminution of chloride and sodium is not brought about by an increase of these substances in the stomach, as might be anticipated from the work of Gamble and McIver (18) on pyloric obstruction in rabbits. Although some of the diphtheritic rabbits passed a few loose stools containing mucus, it was not felt that the diarrhea was sufficient to explain the changes in serum electrolyte. Retention of water was apparently not the cause of the reduction in concentration of electrolytes, since marked loss of weight occurred in all toxic animals. While the water intake was not measured, the animals seemed to drink freely except during the day before death.

Since the decreases in sodium and chloride were approximately equal, base deficit does not explain the low bicarbonate. Although increase of inorganic phosphate replaces part of the bicarbonate, the diminution of bicarbonate must be considered, by exclusion, as due chiefly to accumulation of organic acids and possibly sulfate. A few specimens of serum were distilled into Scott-Wilson (19) reagent but no ketone bodies were detected. Undoubtedly lactic acid accounts for part of the diminution of bicarbonate but the previous studies (1), while indicating a probable increase in lactic acid, were not convincing, because of the difficulty of obtaining from normal rabbits blood specimens which represented the resting state.

SUMMARY

The serum electrolyte and hemoglobin concentration of fasting rabbits after intravenous injection of diphtheria toxin is reported. The diphtheritic rabbits showed the following changes: (1) progressive increase in hemoglobin concentration; (2) approximately equal decrease of sodium and chloride; (3) decrease in bicarbonate; (4) marked increase in serum inorganic phosphate which paralleled the severity of the intoxication.

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STUDIES OF THE HEART AND CIRCULATION IN DISEASE; ESTIMATIONS OF BASAL CARDIAC OUTPUT, METABOLISM, HEART SIZE, AND BLOOD PRESSURE IN 235 SUBJECTS

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In a previous communication the results of duplicate estimations of cardiac output on 50 persons, the majority hospital patients in the basal condition, have been reported (1). The opportunity to extend this investigation rapidly was provided by the development, by Donal and Gamble, of a physical method for the estimation of ethyl iodide by means of its thermal conductivity in a katharometer (2). This improvement so increased the rapidity with which the cardiac output could be estimated, that about two hundred hospital cases, four hundred estimations of cardiac output, were added to the series with the expenditure of less time and effort than had been required to secure the results in the first fifty cases by the chemical technique. When the new series was combined with the old, over 200 cases were secured in which satisfactory estimations of basal cardiac output, metabolism, blood pressure, and pulse rate, had been made on resting patients 15 or more hours after the last meal; and in which orthodiagrams had been secured also. The analysis of these results forms the subject of this paper.

As soon as results, secured by any cardiac output method, are examined a difficulty appears which can best be set forth by an example. The average cardiac output of 31 healthy persons is 2.9 liters per minute per 100 pounds, that of 8 cases of anemia 3.2 liters; should the difference be considered significant or not? An estimation of the validity of differences is based on knowledge of the relative accuracy of the methods involved. One may try to ascertain the accuracy of a cardiac output method, when applied to man, by the agreement of duplicate estimations, and by comparison of the results with those obtained by other methods, preferably based on different physiological principles. But it should be emphasized that cardiac output procedures have not attained the position of those methods the accuracy of which can be tested by estimation of known quantities. Therefore, we have fallen back on another way of approaching the problem and have estimated the significance of our differences by statistical procedures.

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By this means normal standards have been established for various functions of the circulation: viz, the cardiac output per minute, per body surface (cardiac index) and per body weight; the cardiac output per beat (stroke volume) ; the arteriovenous oxygen difference; the work of the left ventricle per minute and per beat; and the relationship of these functions to the size of the subject and of his heart.   Knowing the normal statistics, one is then in a position to decide whether the circulation in diseased conditions is significantly different.

But the plan of the investigation was not limited to the description of the circulation in common diseased conditions, a field in which considerable progress has already been made (3).   It was also hoped to discover a physiological relationship by which the condition of the heart muscle could be ascertained.   This has been found in the relationship between the heart's work and its size, an extension of Starling's Law of the Heart to clinical conditions.   This criterion has provided a physiological estimate of the condition of the myocardium in the common clinical conditions to which our cardiac output method could be properly applied, and has permitted a clearer differentiation between disease of the heart and disease of the peripheral circulation in these conditions.

METHODS

All patients were lying at rest 15 or more hours after the last meal.   Outpatients lay down for over an hour before the first estimation.   Ward patients were brought to our room in bed or in a wheel-chair, and then lay down for ½ hour or longer before starting the determination.

The use of the katharometer for the analysis of ethyl iodide has not introduced any material change in the conduct of the cardiac output estimation (4). We made a few minor changes during the course of the investigations in order to increase the margin of safety.   The rebreathing bag was filled with air containing the concentration of ethyl iodide expected at equilibrium.   In place of the old average distribution coefficient (6.1) for ethyl iodide in air and blood, we made use of the data of Cool, Gamble and Starr (5) and estimated the distribution coefficient from the subject's red blood count.   The results of cardiac output estimations published before (1) were recalculated and appear in this paper slightly changed, 61/66 and 61/64 of the original value for men and women respectively.   In cases of anemia, of diabetes, and in certain other instances, 34 cases in all, Dr. Cool determined the distribution coefficient of the subject's blood, and his result was utilized in calculating the cardiac output.

The estimations of basal metabolic rate were made by the analysis of expired air.   In calculating the result the average value of the respiratory quotient was assumed, but we made use of the calculated quotient to discard a few estimations in which it was unreasonable.   Pressure of work made us omit the duplicate estimation of metabolism, in many cases.   The single determination was made during the second estimation of cardiac output.   Therefore, when the relationship between the metabolism and cardiac output was studied the result of the second estimation has been used.   In all other instances the two calculated cardiac outputs were averaged.

The graphic record of respiration was omitted, reliance being placed on reading the spirometer every minute and counting the respiratory rate.

The heart volume was determined by means of the orthodiagraph, the silhouette area and anteroposterior diameter being determined. The volume was calculated by means of Bardeen's formula (6); 0.53 (silhouette area)$^{\frac{3}{2}}$. After the investigation had been completed we recalculated the heart volume of a majority of the cases according to the newer method of Kahlstorf (7); 0.63 (silhouette area $\times$ maximum anteroposterior diameter). But we had not fulfilled the exact requirements of that author, our silhouette areas having been determined with the arms down while he specifies that the arms should be raised. A few experiments to determine whether this made much difference indicated that the silhouette area might be changed as much as 5 per cent. In any event there was not enough difference between the results of the Bardeen and Kahlstorf methods to influence the statistics. In the following discussion the method of Bardeen was employed in all cases in which the method of Kahlstorf is not specifically mentioned.

We have continued to make orthodiagrams with the patient standing. We would have preferred to estimate the heart size with the subject lying, and some difference in the two positions is to be expected (6). But, we became convinced that the higher diaphragm shadow in recumbent patients would so reduce the accuracy with which the cardiac silhouette could be determined, that we decided to utilize a position different from that in which the other data were secured.

The katharometer was tested 25 times, usually at intervals of 10 days, during the course of this investigation. When known dilutions of ethyl iodide were estimated under the conditions present in cardiac output determinations, it never failed to give satisfactory results. Minute leaks, introduced intentionally, could be detected at once by the behavior of the mercury pressure gauge. An accidental leak was encountered only once during these experiments.

The analyses pertaining to cardiac output were all performed by Donal, those concerned with metabolism by Shaw. The orthodiagrams were all made by Margolies. The selection of the patients was largely made by Starr and Collins; most of the cases came from wards under their care. Margolies selected a few from the Out-Patient Department. Starr, Collins or Gamble supervised the subjects during the determinations and made clinical observations.

The calculations incident to the statistical analysis were made by Starr and Donal, each checking the other. We are again indebted to Dr. W. C. Stadie for suggestions and criticism of this analysis.

NOMENCLATURE AND METHODS EMPLOYED IN THE STATISTICAL ANALYSIS

Of the articles on statistics consulted, that of Dunn (8) proved most useful, and the methods there described have been followed.

To facilitate the calculations, the data were converted into smaller units by selecting an arbitrary origin near the midpoint and dividing the range of values into ten or more class units. The computations were made from frequency distributions and class units, and then converted back into the original units.

Differences have been considered significant when the probability of their arising from the sampling is less than 5 in 100. Throughout this paper the differences mentioned without qualification meet this test.

We have assumed that the relationships that we have studied were linear. The scattering of the data seemed too great to warrant any other method of procedure.

The term *cardiac output* has been employed to mean the output of one side of the heart per minute, as is the custom. The *cardiac index* has come to mean the cardiac output per minute, per square meter of body surface. The *stroke*

volume is the cardiac output per beat. We have employed the term *cardiac work* to mean the work of the left ventricle per minute.

As the subjects varied greatly in size, from 85 to 265 pounds, the absolute values of the cardiac output, stroke volume, and cardiac work have little meaning. It has been the custom to compare these figures with the body surface area. For reasons which will be discussed later, we prefer to refer them to the body weight. For convenience of expression reference to the latter will be omitted from the text when the full term appears in the table under discussion.

In Table VI the capacity of certain cardiac cases to perform muscular work has been indicated by the nomenclature devised by the New York Heart Committee, viz.: Class I, no restriction of activity; Class IIa, slight restriction; Class IIb, marked restriction; Class III bed patient.

SELECTION AND GROUPING OF THE CASES

Patients with congestive failure, with advanced pulmonary abnormalities, with emphysema, or with fever were avoided. We have employed the criteria given before (1), failure to attain a basal metabolic rate, hyperventilation, etc., to eliminate 25 cases from the statistical analysis. The remainder have been divided into clinical groups shown in Table I. The diagnoses of the individual cases can be found in Table VI, except Numbers 1 to 50 which have been reported before (1), under the same numbers. The sex, age, height and weight of these cases, not given before, have been appended in Table VII. Tables VI and VII appear together at the end of this paper.

TABLE I

Arrangement of the clinical material

Cases	Subgroup	Group
31 healthy persons 47 hospital patients with normal circulations (14 cases of hypotension also in above 2 groups)	Normal circulation group 78 cases	Control group 140 cases
28 cases of hypertension 7 cases of anemia 15 cases of hyperthyroidism 13 cases of neurocirculatory asthenia 6 miscellaneous cases	Abnormal circulation group	
20 cases—*Myocardial Disease Group*	Cases once in congestive failure, now having recovered compensation	Cardiac group

8 cases of valvular disease with regular rhythm
12 cases of arrhythmia
  A. 8 arrhythmic when tested
  B. 8 in regular rhythm when tested
10 cases of coronary disease
5 cases of acute endocarditis
	Cases never in congestive failure	

5 cases of aneurysm of the aorta

Into the *control group* (Table I) we have gathered all cases believed to have normal myocardial function. These were selected more rigidly than in our previous study (1). In addition to those who had once been decompensated, all cases with any form of organic cardiac disease, serious arrhythmia, angina pectoris, or aneurysm were excluded. In addition, we thought it wise to omit 5 cases of hyperthyroidism, one case of anemia in shock, and one of myxedema, because of doubt concerning the condition of their hearts; and these cases have been starred in Table VI. On the other hand, we included cases of hypertension with cardiac enlargement in the control group, if their general condition was satisfactory.

Of the subdivisions of the control group, the *healthy group* was drawn from the faculty, students and technicians of the Medical School. The *patients with normal circulation* were taken from the hospital wards, a few older patients with slight sclerosis of peripheral vessels and three with peripheral vascular disease, but without other evidence of circulatory disease, were included. Together, these two groups form the *normal circulation group*. Cases with systolic blood pressure over 150 mm. Hg, except cases of hyperthyroidism and aortic regurgitation with large pulse pressure, have been included in the *hypertension group*. All cases having less than 70 per cent of hemoglobin were classified as *anemias*. The cases of *hyperthyroidism* were identified by their basal metabolic rate. The cases of *neurocirculatory asthenia* were characterized by dyspnea and palpitation on slight exertion, and in addition several of the young women complained of transient cardiac pain, not definitely related to exertion. The diagnoses of the individual cases may be found in Table VI.

Of the cases in the normal circulation group, fourteen had a systolic pressure less than 100 mm. These have been combined as the *hypotension group*, in order to contrast the findings with those of cases of hypertension.

The *cardiac group* consisted of cases with undoubted cardiac disease, but the conditions included were too diverse to warrant consideration of the group as a whole. The most important subdivision was the *myocardial disease group*, patients who, though now compensated, once had congestive failure. Therefore, there can be no doubt that they had serious myocardial disease at the time of the tests. The cases in the other subdivisions had never been decompensated and the clinical estimate of their myocardial condition did not seem sufficiently definite to base a classification upon it. For this reason, the chronic cases have been grouped according to their outstanding features: *valvular heart disease, arrhythmia,* or *coronary disease* (angina pectoris or occlusion). The cases believed to have *active endocarditis* at the time of testing were grouped separately, as were the cases of *aneurysm* demonstrable by x-ray.

It is likely that medication, especially digitalis (14), influenced heart size and cardiac output in cases receiving it. We have made no attempt to correct for this variable, but, when cases were receiving such drugs, the fact has been set forth in Table VI.

RESULTS AND DISCUSSION

Deviation of duplicate estimations of cardiac output made on the same day

In thirty healthy and intelligent subjects the average deviation of the duplicate estimations from their mean was 3.45 per cent. For the first 154 patients tested this deviation was 6.45 per cent. This is a significant difference. It cannot be attributed to training as the healthy group, with two exceptions, had had no previous experience with our apparatus. We attribute it to the unavoidable nervousness that the more ignorant subjects feel when undergoing an unusual experience. The results show that standards of variation, secured on intelligent subjects, cannot be applied directly to hospital patients. In the former the deviation of duplicate estimations will exceed 10 per cent only once in twenty times; in the latter 20 per cent will be exceeded once in twenty times.

Deviation of estimates secured on the same subject on different days

Our data on this important point has been presented in Table II. The individual results obtained on any day have been used to find that day's average. This figure has been used as the basis of studying variations from day to day, the intervals reaching a year in some cases. The mean deviation for the 15 subjects is 6.3 per cent. The expectation is that, when the results secured on two days are compared, they will deviate from their mean by less than 20 per cent in 19 cases out of 20. But while some subjects (Number 48) are characteristically steady from day to day, and on the same day, others (Number 43) are far more variable both in basal cardiac output and metabolism; and this difference has persisted over the period of 7 years since our method was developed.

Discussion of mean values

The mean values of the various groups and the standard deviation of the data about the mean are shown in Table III.

Before these data (Table III) could be interpreted it was necessary to ascertain the effects of *sex* and *age* upon the cardiac output. This has been estimated on the 78 cases composing the normal circulation group. The average cardiac index for males and females was identical. In the period before 20 years the average cardiac index is higher than at any time later, after 50 it slowly declines but the number of cases was too small to demonstrate the significance of the difference (Figure 1). If the cardiac output per kilo is considered, the increase in the average before the age of 20 is still more striking. The general trend is very similar to changes of the basal metabolic rate with age and this curve, prepared by DuBois (9) has been appended to Figure 1. Therefore, our normal standards derived from a group of diverse ages are not applicable to persons under 20 years without correction. The changes in advanced age have not been sufficiently defined

to make correction possible.   All but 10 of our 235 subjects were over 20 years of age, and when these were omitted it made little difference to the averages, but considerable difference to some of the correlation coefficients. In the data to be discussed the cases under 20 years of age have always been included unless a statement to the contrary is made.

TABLE II

Basal cardiac outputs on the same subjects on different days

Subject number	Date and cardiac output	Average deviation of each days average about the mean
	liters per minute	*per cent*
48	2-13-28, 3.6; 3-13-28, 3.8, 3.7, 3.8; 3-17-28, 3.7, 3.6, 3.6; 1-16-30, 3.3, 3.3; 4-30-31, 4.2; 5-8-31, 3.7; 3-18-32, 3.5; 3-21-32, 3.7, 4.0, 3.5, 3.4; 3-22-32, 3.9, 3.8, 4.1, 4.2; 9-25-33, 3.8, 4.0; 9-28-33, 3.2, 3.3; 1-31-34, 3.9, 3.9;	2.8
43	3-8-28, 4.9, 4.5, 3.8; 3-15-28, 3.1, 4.3, 3.1; 4-24-28, 2.8, 2.7, 3.2; 1-18-30, 4.5, 4.9; 5-17-32, 3.0, 3.1; 11-3-33, 5.1, 4.7;	9.2
51	4-18-32, 3.0, 2.9, 3.0, 3.0; 12-6-33, 4.1, 3.9;	14.7
52	4-21-32, 5.1, 4.1, 4.2, 4.3; 6-23-33, 5.0, 5.3;	7.6
53	5-10-32, 3.7, 3.9, 3.7; 6-17-32, 3.8, 4.0;	1.8
8	5-28-31, 2.1, 2.2; 3-28-32, 2.1, 2.2, 2.6;	1.7
151	3-14-33, 2.6, 2.5; 3-24-33, 2.2, 2.4;	5.2
124	2-10-33, 3.7, 3.6; 3-25-33, 3.1, 3.0;	9.0
152	3-17-33, 4.5, 5.1; 6-25-33, 4.5, 5.1; 10-30-33, 3.9, 4.2;	3.7
220	6-23-33, 3.3, 3.8; 9-20-33, 2.7, 2.6;	14.5
223	6-25-33, 3.4; 10-26-33, 4.5, 4.4;	12.2
227	10-27-33, 3.2, 3.4; 11-24-33, 3.4, 3.1;	0.8
A	2-6-31, 3.3, 3.2; 7-15-31, 3.1, 2.9;	4.0
B	3-4-31, 2.0; 3-10-31, 2.0, 2.0;	0
C	4-4-31, 2.3; 4-6-31, 2.4; 4-10-31, 1.7;	6.8
	Average	6.3

TABLE III

Statistics concerning the heart and circulation under basal conditions
Means (in Roman type)
Standard deviations about the means (in italics)

	Approximate number of cases	Cardiac output	Cardiac output	Cardiac stroke volume	Arterio-venous oxygen difference	Left ventricular work per minute	Left ventricular work per beat
		liters per minute per sq. meter body surface	*cc. per minute per kgm. body weight*	*cc. per kgm. body weight*	*cc. per liter blood*	*gram-meter per kgm. body weight*	*gram-meter per kgm. body weight*
Healthy persons...	31	2.40 *0.55*	64.6 *15.3*	0.99 *0.28*	58.8 *14.0*	80.3 *18.5*	1.21 *0.29*
Patients with normal circulations.	47	2.15 *0.71*	61.6 *21.6*	0.86 *0.28*	63.9 *19.5*	74.8 *26.9*	1.06 *0.33*
Anemia..........	7	2.52 *0.67*	71.5 *19.9*	0.83 *0.14*	48.2 *12.6*	81.1 *29.1*	0.97 *0.26*
Hyperthyroid.....	15	2.90 *0.89*	81.6 *27.6*	0.80 *0.25*	70.1 *20.6*	113.6 *33.1*	1.14 *0.35*
Hypertension.....	28	2.19 *0.81*	57.2 *28.2*	0.70 *0.29*	66.1 *20.0*	113.5 *59.1*	1.43 *0.65*
Hypotension......	14	2.39 *0.66*	68.4 *27.4*	0.89 *0.23*	65.7 *22.3*	73.2 *28.2*	1.04 *0.36*
Neurocirculatory asthenia........	13	1.70 *0.47*	50.0 *7.3*	0.62 *0.19*	80.8 *19.3*	60.6 *15.6*	0.76 *0.28*
Patients recovered from congestive heart failure....	20	1.74 *0.41*	45.5 *10.1*	0.55 *0.18*	82.0 *20.6*	71.0 *22.5*	0.88 *0.33*
Coronary disease..	10	2.22 *0.85*	58.2 *25.1*	0.74 *0.26*	69.0 *27.9*	86.0 *39.7*	1.09 *0.45*
Valvular heart disease..........	8	2.13 *0.37*	68.4 *22.9*	0.89 *0.31*	63.0 *23.0*	91.1 *36.4*	1.31 *0.49*
Formerly in arrhythmia.........	8	2.15 *0.45*	55.2 *18.3*	0.66 *0.20*	78.7 *18.3*	81.4 *26.7*	0.92 *0.31*
Acute endocarditis	5	2.47 *0.40*	64.8 *5.1*	0.85 *0.12*	64.5 *13.6*	107.0 *12.8*	1.05 *0.26*

The *healthy group* was, composed of persons whose health and fitness were probably superior to the average and almost all were accustomed to laboratory procedure.   The hospital *patients with normal circulations* were not only untrained, but suffering from the conditions indicated in Table VI,
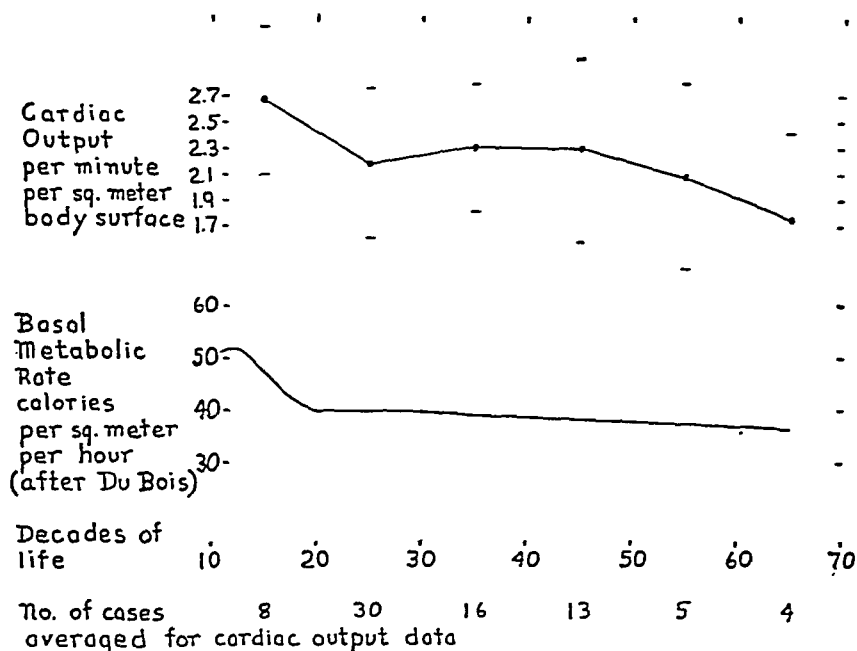
FIG. 1. EFFECT OF AGE ON BASAL CARDIAC OUTPUT

The results obtained on persons in each decade of life have been averaged. The dots indicate the means of these groups, the dashes the standard deviation about the means.   The curve showing the effect of age on basal metabolic rate (after DuBois) has been appended for comparison.

many were in poor physical condition.   The former group averaged 5 years younger and 20 pounds heavier than the latter.   In spite of these differences the healthy subjects had an average cardiac output, arteriovenous oxygen difference, heart work, and heart size which did not differ significantly from those of the hospital patients.   But the average stroke volume and heart work per beat were smaller in the hospital cases.   These differences were small and barely attained significance.   Whether they demonstrate a real effect of intercurrent disease on the circulation or were merely due to increased pulse rate from nervousness in the more ignorant patients, we have no means of deciding.   But it seemed evident that the results obtained on the hospital patients with normal circulations would make the better standard for comparison with those secured on other patients, so we have used these statistics as a basis for ascertaining differences from the normal.

The *anemic patients* had an average hemoglobin of 50 per cent. They differed from the normal in that their arteriovenous oxygen difference was smaller. Their stroke volume was normal, and the pulse rate being faster, the average cardiac output was above normal, but we have not demonstrated that this difference is significant. This increased cardiac output in anemia has been demonstrated by others (3).

When the ethyl iodide method is employed in the investigation of *hyperthyroidism,* a difficulty presents itself. A slow lowering of basal metabolism often follows the administration of iodides to such patients, and the inhalation of ethyl iodide might depress the metabolism, and perhaps the cardiac output also, at the time the determinations were made (10). Seeking for such an effect, we compared the results of routine estimations made by the Benedict-Roth apparatus with those secured during the inhalation of ethyl iodide. The results indicated that the latter procedure had no immediate effect on the metabolism of our cases. This may have been due to the fact that all but two of them had been receiving iodide by mouth before the estimations were made, and the maximum iodide effect had been secured already. ·

The average basal metabolic rate of the hyperthyroid group was 45 per cent above the calculated normal. The group had a more rapid pulse rate, a larger cardiac output and a greater cardiac work than the normal. No other differences from the normal have been demonstrated to be significant. The increased cardiac output in hyperthyroidism is well known (3).

The cases of *hypertension* had large hearts which performed an increased amount of work both per minute and per beat and had a smaller stroke volume than the normal. In other respects they resembled the normal group. The cases of *hypotension* had circulations not significantly different from the normal, but their results showed a marked contrast with those secured in hypertension.

The results obtained in cases of *neurocirculatory asthenia,* though suggested in previous work (1), surprised us greatly. Although the symptoms occurred chiefly on exertion, the circulations at rest were highly abnormal. The average cardiac output, stroke volume, and heart work per minute and per beat were all far below normal. The arteriovenous oxygen difference was abnormally large, so these patients doubtless had a lowered oxygen tension in their tissues. Except for the size of their hearts, smaller than normal, their averages closely resemble those secured on patients with undoubted myocardial disease.

Of the cardiac group, those with undoubted *myocardial disease,* who were once decompensated, stand out. The averages show that these patients had an abnormally small cardiac output, stroke volume, and work per beat; an abnormally large arteriovenous oxygen difference, and very large hearts. More unexpected was the similarity of their average heart work with that of the patients with normal circulation. This may be interpreted

as indicating that the methods of compensation for myocardial disease, cardiac enlargement, increase in rate, etc., sufficed to bring the work performed by the heart back to normal, but this was insufficient to restore the circulation to its usual level. This normal average work per minute is largely due to the presence of cases of hypertension in the myocardial disease group. If these are omitted the average is much lower.

In the *coronary group* are 7 patients diagnosed as having had coronary occlusion. The electrocardiograms showed characteristic changes (11). None was in an acute attack when the estimations were made. In four, the attack had occurred within six weeks and they had not yet left their beds. Two of these, Numbers 31 and 88, died shortly after the determinations, and the necropsies showed both old and fresh infarcts in each. Two cases of angina pectoris are also included in the group, and three of the cases of occlusion had previously exhibited this symptom. The most interesting aspect of the group averages is the close agreement with the normal in all items except heart size. It would appear that the effect of the lesions, probably involving only a small fraction of the total myocardium, has been amply compensated by the slight cardiac enlargement, as far as can be judged from studies on the circulation at rest.

In cases in which *valvular disease* was the dominant finding, and which had never been decompensated, the average values of their resting circulations were never significantly below normal. Their hearts were larger than normal and this had apparently compensated for the valvular defect, as far as the resting circulation was concerned. In a few individuals the results were far below normal, and their future course will be followed with interest.

The cases, the outstanding feature of which was attacks of *arrhythmia*, and which were *in normal rhythm* when the test was made, form a homogeneous group. The average arteriovenous difference and stroke volume resembled that of the myocardial disease group; they were significantly different from the normals. Their hearts were larger than those of the patients with normal circulations, but the difference is not significant. The other means (Table III) are close to the normal.

The cases *in arrhythmia* when tested were too diverse clinically to warrant statistical analysis as a group, but the individual cases demand some comment. They included five cases in auricular fibrillation, three in paroxysmal tachycardia, and two in complete heart block. The first were under the influence of digitalis, and except for the large size of their hearts, the findings do not differ significantly from the normal, except in Case 55, who died within 6 months of the test. Two of the cases of paroxysmal tachycardia exhibited a highly abnormal circulation during the attack, but the third maintained his circulation within normal limits, and indeed he suffered little inconvenience. The two cases of complete heart block were characterized by a small output per minute in spite of the abnormally large stroke

volumes. The most interesting finding in these cases was the extremely low basal metabolic rates, —40 and —52 per cent. These figures were so low that we suspected an error. But a duplicate estimation on Case 234 checked well, the respiratory quotients were normal in both cases, and no such low result was obtained on any other patient in this series. These cases might be thought of as having maintained a normal arteriovenous oxygen difference, and hence a normal oxygen tension in their tissues, by reducing their metabolic rate and so compensating to some degree for the deficient circulation.

The cases of *active endocarditis,* 4 with acute rheumatic fever and one with subacute bacterial endocarditis show averages which are not significantly different from the normal, except for the work per minute which was above normal.

A general *survey of the averages* (Table III) impresses one with the constancy rather than the variability of many physiological functions in disease. The average heart volume in the myocardial disease group differs from the normal by 115 per cent, but the average cardiac output and its related functions never differ from the normal by more than 36 per cent. Compared with the scattering of the data, the differences between the means seem small. The overlapping is so great that there seems little chance of developing a method to determine the normality of the heart of a given case by means of the cardiac index, cardiac output per kilo, stroke volume, or arteriovenous oxygen difference.

Figure 2 shows that, in some of the clinical groups, the greatest frequency coincides, not with the group's mean, but with the mean of the normal persons. The hypertension group is the most striking example of this tendency. This lack of the normal statistical distribution of the results indicates that the group has lost its unity. It permits us to think of it as consisting of two parts: first, patients who have maintained their heart's work at the normal value, by reducing their cardiac output below normal and sacrificing the oxygen tension in their tissues; and second, those who have maintained a normal circulation at the expense of cardiac hypertrophy and increased cardiac work (1). One might expect that cardiac failure would eventually supervene in the latter and that breakdown would occur elsewhere in the former group.

Our average values for the cardiac index and arteriovenous oxygen difference in the normal circulation group agree closely with those obtained in normal persons by other cardiac output methods (3).

Discussion of relationships

The ideal relationship to distinguish normal from abnormal myocardial function would be one which holds closely, not only for normal conditions, but also for those in which the circulation, but not the heart, is primarily affected. Therefore, taking relationships which were significant for per-
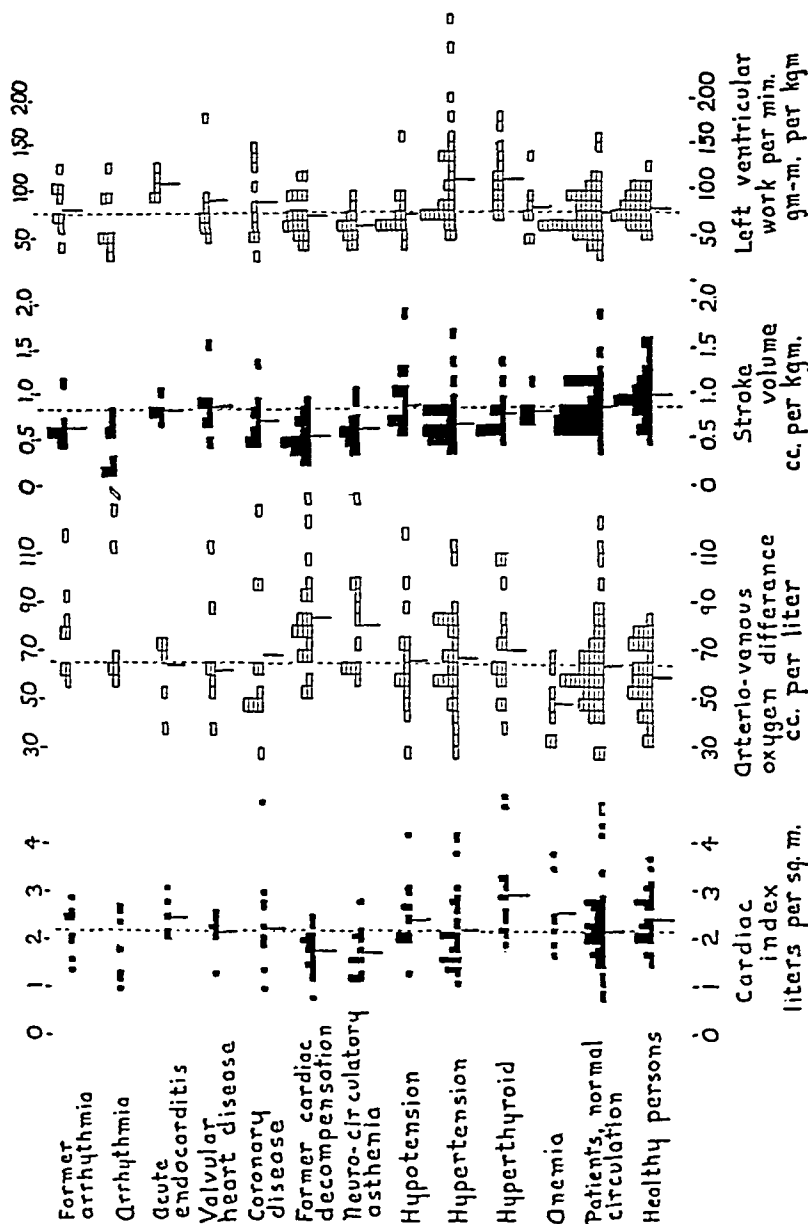
FIG. 2. FREQUENCY DIAGRAMS

The values obtained on individual subjects, have been grouped in accordance with class units into which the range of values has been divided. These class units are: cardiac index, 0.1 liter per square meter; arterio-venous oxygen difference, 5 cc. per liter; stroke volume, 11 cc. per kilo; left ventricular work per minute, 11 gram-meters per kilo. The range of values covered by the cardiac index having been divided into smaller units, the squares representing the individual cases in this group are narrower than the rectangles employed for the other groups. The height of all the units is the same, and may be ascertained from those outlying. When a value occurs more than once the symbols have been piled one on top of another. The means of the groups have been indicated by a bar extending below the base line. The mean values secured on the patients with normal circulations have been extended throughout the data by a dotted line to facilitate comparison.

sons with normal circulations we planned to test them, first in cases of hypertension, and finally in the entire control group, which included those with normal as well as those with abnormal circulations.

TABLE IV
Correlation coefficients and their standard errors

Relationships, under conditions of basal metabolism	Normal circulation group—78 cases		Hypertension group—28 cases		Control group 137–140 cases	
	Correlation coefficient	Standard error	Correlation coefficient	Standard error	Correlation coefficient	Standard error
Level of correlation coefficient below which there is no significant correlation..........	0.28		0.38		0.17	
Oxygen consumption per minute and body surface area............	0.65	0.068				
Cardiac output per minute and body surface area...............	0.36	0.100	−0.26	0.18	0.18	0.083
Cardiac output per minute and body weight...	0.44	0.089	−0.22	0.19	0.22	0.081
Cardiac output per minute, and oxygen consumption per minute..	0.40	0.098	0.46	0.16	0.45	0.069
Cardiac output per minute and heart volume..	0.38	0.099	0.27	0.18		
Stroke volume and heart volume.............	0.44	0.094	0.56	0.13	0.42	0.072
Left ventricular work per minute and heart volume...............	0.42	0.096	0.44	0.16	0.57	0.058
Left ventricular work per beat and heart volume.	0.50	0.097	0.69	0.10	0.67	0.047

In the interpretation of the results (Table IV), and especially in the comparison with correlation coefficients obtained previously (1), several facts must be kept in mind. The correlation coefficient considered alone does not fully represent the degree of correlation of the data, i.e., a coefficient of 0.8 does not necessarily represent more perfect correlation than one of 0.7. To compare the correlation one must consider not only the coefficient but also the number of cases in the series. This has been given (Table IV) as a level, varying with the number of cases, below which there

were more than 95 chances in 100 that there was no correlation at all (12). In Table IV we have also given another expression based on the coefficient and the number of cases, the standard error of the correlation coefficient. If the coefficient is twice its standard error, the probability of the data being associated is 95 in 100, and this is the usual test of significance. If this ratio is 3 to 1, this probability is over 99 in 100. Thus the degree of correlation of the data may be ascertained by comparing the coefficient with its standard error. When these facts are kept in mind it becomes evident that, although the coefficients given in Table IV are sometimes lower than those obtained before (1), the evidence for the validity of the relationships has been greatly strengthened by the addition of the new data.

Basal metabolic rate and body surface area. This relationship is well known. In our subjects with normal circulations its correlation coefficient is 0.65. This, nine times its standard error, indicates a high order of association. It will be convenient to compare the other relationships with this well known one, and for convenience of expression, it will be referred to as the *basal metabolic relationship* hereafter.

Basal cardiac output per minute and body surface area. This is generally known as the *cardiac index,* and it is the usual method of reporting the results of cardiac output estimations. In normal persons it easily passes the test of significance; therefore, this conception is valid, but the correlation is far less than in the basal metabolic relationship. When persons under 20 years of age are omitted, the correlation in the normal circulation group is improved, from 0.36 to 0.41, but it is still significantly smaller than in the basal metabolic relationship. Among cases of hypertension there is no correlation between cardiac output and body surface. In the whole control group, which includes the cases of hypertension, the correlation is barely above the level of significance. Therefore, the cardiac index is likely to prove a poor tool for the detection of heart disease. It does not distinguish disease of the heart from that of the circulation.

Basal cardiac output per minute and oxygen consumption. The quotient of these items gives the arteriovenous oxygen difference, and its importance has been properly emphasized (3). In normal persons the correlation is about the same as the preceding, it is much inferior to the basal metabolic relationship. Unlike the cardiac index, omission of the cases under 20 years of age has but little effect, and also, it holds for cases of hypertension. Its correlation is far superior to that of the index in the control group, attaining a value which, while considerably lower, is not significantly different from the basal metabolic relationship. Therefore, we regard this relationship as better and more fundamental than the cardiac index. An additional reason for this belief follows.

Partial correlations in the preceding relationships. Having calculated the correlations for the three interlocking relationships: oxygen consumption and body surface area, cardiac output and oxygen consumption, and

cardiac output and surface area for the normal circulation group, it is possible to make use of the statistical device known as partial correlation (8). This permits holding one of the variables constant by mathematical means and studying the resulting effect on the relationship between the other two. Thus, if the oxygen consumption is held constant we find that the partial correlation coefficient between the cardiac output and body surface area is 0.012, i.e., there is no significant correlation between them. Obviously, therefore, the cardiac output in normal persons is related to the body surface only because it is related to the oxygen consumption which in turn is related to the surface area. If a group of patients could be secured with identical oxygen consumptions we predict that their cardiac outputs would not be related to their surface areas at all.

Cardiac output per minute and body weight. This proved to have a coefficient considerably higher than the cardiac index but the difference, though suggestive, is not statistically significant. Like the index, this relationship is different for persons under 20 years of age, the correlation in the normal circulation group improving from 0.44 to 0.50 when such cases are omitted. In Table III it will be seen that the mean cardiac output per kilo, of the healthy group, is closer to that of the hospital patients with normal circulations, than is the mean cardiac index. None of these differences are statistically significant; therefore, we are not in a position to decide definitely whether body weight or surface is preferable. But what evidence we have suggests that cardiac output should be compared with body weight rather than surface. Neither relationship holds well among the cases with abnormal circulation, neither can be used to detect cardiac disease.

Stroke volume and heart volume also *output per minute and heart volume.* In the normal circulation group these relationships have a correlation quite similar to the cardiac index. The former has a higher correlation in the hypertension and control groups. Both are inferior to the basal metabolic relationship.

Left ventricular work per minute and heart volume. In the control group this relationship is superior to those involving cardiac output, but, being inferior to that which follows, although not significantly so, it needs little discussion.

Left ventricular work per beat and heart volume. This is obviously the most interesting relationship that we have discovered. Among the patients with normal circulation it has the highest correlation except for the basal metabolic relationship. The differences in this group are not significant, but they become so as soon as the relationships are tested against patients with abnormal circulations. Under these conditions this relationship holds so well that its superiority over the others is very obvious. Indeed, in the control group, its coefficient is higher than that of the basal metabolic relationship, but the difference is not significant. This confirms

our previous conclusion (1). It demonstrates that the basal work per beat of the normal heart is a function of its size, an extension of Starling's Law of the Heart (13) to clinical conditions.

When the heart volumes were calculated by the method of Kahlstorf instead of that of Bardeen the correlation of this relationship in the control group remained the same.
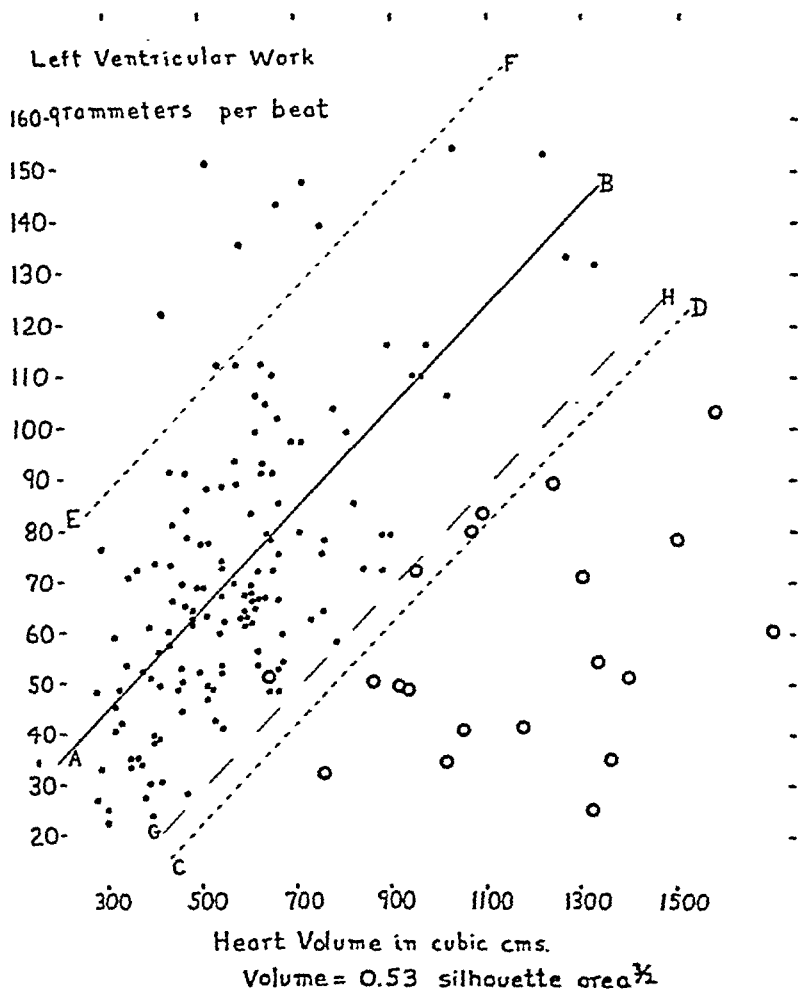
FIG. 3. LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME

Each symbol represents the average of duplicate estimations. The *dots* indicate the values obtained in the *control group*. The solid line AB is the calculated best line for this group (the regression of heart work on volume). Lines CD and EF have been placed at a distance of twice the standard deviation about AB. Therefore, if any result falls to the right of CD the probability of its differing from the normal is about 97.5 in 100. The probability that a value falling to the right of GH differs from the normal is 95 in 100. The *circles* indicate the values obtained on persons who had *formerly had cardiac decompensation*.

Figure 3 shows the relationship between the left ventricular work per beat and heart volume for a large part of the data. The line AB is the calculated best line for the control group, the regression of work on volume. The lines CD and EF, placed at a distance from AB of twice the standard deviation about the regression line, would enclose about 95 per cent of all similar " control " cases. Therefore, the probability is about 97.5 in 100 that any result falling to the right of this area was secured from a case with abnormal myocardial function. It will be seen at once that the great majority of results, secured on persons who were once decompensated, lie well outside the normal zone. A few have attained the edge of it, but with one exception their chances of belonging to the normal group are less than 5 in 100.

This relationship in different clinical conditions can be further studied (Figures 4 and 5). The lines defining the statistical limits of the control group, shown in Figure 3, have been left in place. The cases omitted from the control group because of the fear that they were on the road to myocardial failure have been circled. It will be seen that in some cases our fears were groundless, but the majority of the values omitted are near the lower limit of the normal range.

The results on cases of hypertension had a poorer correlation than that found before (1), but the difference is not significant. This was chiefly due to three cases with small hearts doing large amounts of work. One of these cases was readmitted to the hospital three months after the test. During the interval the heart had doubled in size and marked hypertrophy was demonstrated at autopsy, the weight being twice the normal. Therefore, the increase of work preceded the enlargement in this instance.

Almost half the cases of thyroid disease gave values which lay close to the lower limit of normality. This is consistent with the well known frequency of cardiac complications in these cases.

Cases of functional heart disease and anemia gave results within normal limits.

We were surprised to find that numerous cases of coronary infarction, two of them later proved to be so at autopsy, gave values near the middle of the normal range. But we made no tests during the period of acute symptoms when a very different result might have been attained. Several cases of angina pectoris were also normal. This has been discussed when the means of this group were considered. The cardiac work-size relationship cannot be used to detect this type of cardiac disease.

The values secured in cases of arrhythmia are also shown in Figure 5. When the same patient was tested in both abnormal and normal rhythm, the results are joined by lines. While some of the values are entirely normal, others are close to the lower limit, and some far outside. The most divergent case died within six months.

Most of the cases of aneurysm gave abnormal values. Perhaps the myocardium had been involved in the luetic process in these cases.

The cases of valvular heart disease who had never been decompensated gave values which fell partly within, partly without the normal. The latter may be thought as being on the road to decompensation; time will tell.
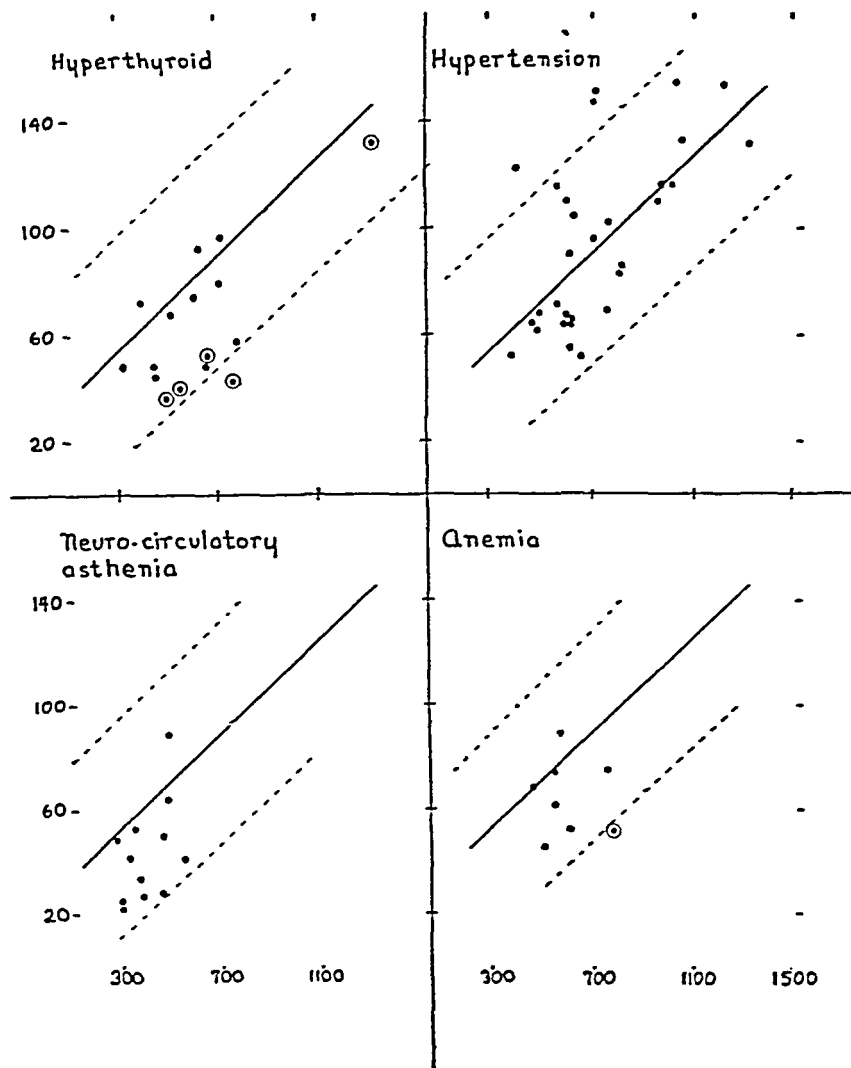
FIG. 4. HEART WORK-VOLUME RELATIONSHIP IN CASES OF CIRCULATORY, BUT NOT CARDIAC DISEASE

The coordinates are heart work per beat and heart volume as in Figure 3. The lines pertain, not to the values here recorded, but to the whole control group, as in Figure 3; they define the normal range of values. The dots representing values obtained in certain cases, in which clinical criteria made us doubtful concerning condition of the heart, have been circled.

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Detection of myocardial abnormality by means of the cardiac work-cardiac size relationship. To determine the normality of a given case according to the newer statistics, one would insert the results into the following equation: 0.052 (cardiac silhouette area in sq. cm.)$^{3/4}$ — (L.V. work per beat in gram meters) = K.

If K is greater than 27, the chances that there is abnormal myocardial function are about 97.5 in 100. If K is greater than 20, the chances of
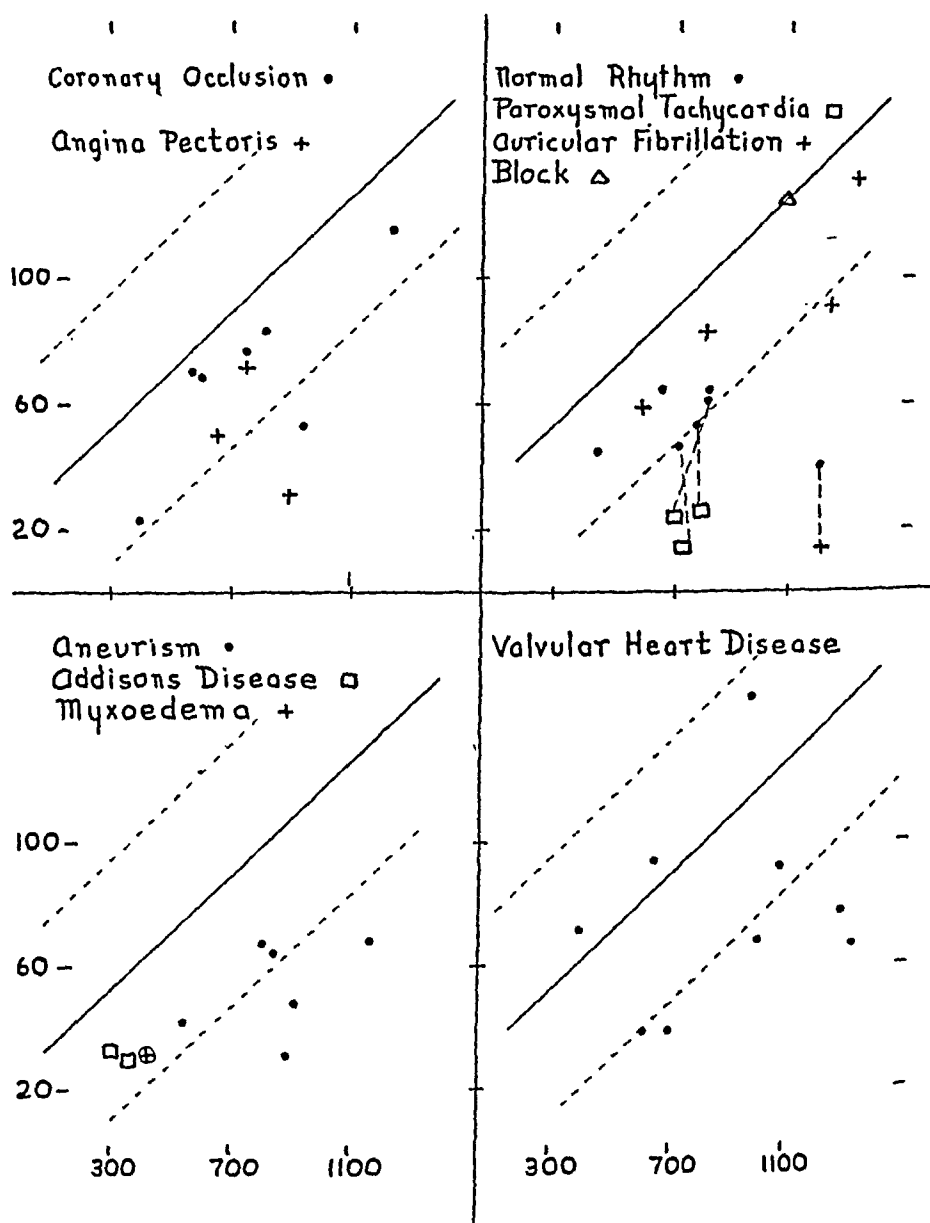
FIG. 5. HEART WORK-VOLUME RELATIONSHIP IN CASES OF CARDIAC DISEASE

A few miscellaneous cases have been included also. Coordinates and lines as in Figure 3 and 4. The values obtained in the same subject during an attack of arrhythmia and in normal rhythm have been connected by broken lines.

abnormality are 95 in 100; if greater than 12 the chances are 90 in 100. The 95 in 100 probability ($K = 20$) divides the cases with undoubted myocardial disease from the control cases in our data, only one of those once decompensated having attained the normal range.

The data obtained in the normal circulation or hypertension groups may likewise be used to define normal myocardial function, but the statistical limits do not divide the normal from the abnormal cases quite as cleanly as the equation given above. The slopes of the best lines of these three groups are very similar and the deviation of the data about them is identical (Table V). This similarity emphasizes the fundamental nature of the relationship between heart work per beat and heart size.

GENERAL DISCUSSION

Our results indicate that the relationship between basal cardiac work and heart size provides a means of defining normal myocardial function and detecting that which is abnormal. This confirms the conclusion of a previous communication (1). The limitations of this means of detecting cardiac abnormality, which were set forth before (1), still hold. Many cases of coronary occlusion or of angina pectoris cannot be distinguished from the normal. In spite of this limitation, it is of interest to apply this criterion of cardiac normality to the conditions which we have studied.

Certain other findings, especially the arteriovenous oxygen difference, may be thought of as demonstrating the condition, not of the heart, but of the general circulation. Since it has been difficult to distinguish between circulatory conditions due primarily to trouble with the heart and those due to trouble elsewhere in the circulation, it is of interest to compare our findings with certain common clinical conceptions.

By our criteria, neurocirculatory asthenia, although often known as " functional heart disease," is shown to be characterized by normal cardiac function but an abnormal circulation. Reflecting on this situation the question might be asked whether the normal heart, confronted by such an abnormal circulation, would not increase its output until the arteriovenous oxygen difference was restored to normal; and whether its failure to do so should not be considered evidence that the heart was abnormal. According to our present knowledge of cardiac physiology this should be answered in the negative. The heart's output, and indeed its work also, is limited by the amount of blood returned to it through the veins. If this is inadequate, no effort on the part of the heart can increase the circulation.

In patients with low exercise tolerance, from either organic or functional heart disease, the circulation at rest has been believed to be normal. Our results show that, on the contrary, the average resting circulation of such patients is distinctly abnormal.

The clinical conception that patients, once in cardiac decompensation,

TABLE V

Equations of the best lines (regression y on x) of the more interesting relationships

Group	Equation	Standard deviations about the regression
Control	Work per beat gm.-m. = 15.1 + 0.0523 (heart silhouette area *sq. cm.*)$^{3/2}$	21.3 *gm.-m.*
Control	Work per beat gm.-m. = 15.8 + 0.0628 (heart silhouette area *sq. cm.* × heart A-P diam. *cm.*)$^{3/2}$	20.9 *gm.-m.*
Hypertension	Work per beat gm.-m. = 27.4 + 0.049 (heart silhouette area *sq. cm.*)$^{3/2}$	21.3 *gm.-m.*
Normal circulation	Work per beat gm.-m. = 25.7 + 0.0431 (heart silhouette area *sq. cm.*)$^{3/2}$	21.3 *gm.-m.*
Control	Work per minute kgm.-m. = 1.27 + 0.00375 (heart silhouette area *sq. cm.*)$^{3/2}$	1.90 *kgm.-m.*
Hypertension	Stroke volume cc. = 23 + 0.0189 (heart silhouette area *sq. cm.*)$^{3/2}$	11.4 *cc.*
Control	Stroke volume cc. = 29.33 + 0.0213 (heart silhouette area *sq. cm.*)$^{3/2}$	16.9 *cc.*
Control	Output per minute *liters* = 0.87 + 0.0132 (oxygen consumption *cc. per min.*)	1.11 *liters*
Normal circulation (all under 20 years of age omitted)	Output per minute *liters* = 0.5 + 0.05 (body weight *kilos*)	1.02 *liters*
Normal circulation (all under 20 years of age omitted)	Output per minute *liters* = −0.38 + 2.4 (body surface area *sq. meters*)	1.09 *liters*

never completely recover from it, is in accord with our results. The one patient (Number 90) who appears to have regained normal myocardial function (Figure 3) was in congestive failure only during two short attacks of auricular fibrillation. The majority of these cases have adapted themselves to a condition in which the average oxygen tension of their venous blood, and hence of their tissues, is considerably below normal.

The foregoing discussion has been presented from the point of view that the relationship between cardiac work *per beat* and heart size is of greater importance than that between work *per minute* and heart size. The latter also separates cases who have once been decompensated from the control group, but there is more overlapping than when the former relationship is employed. Obviously, persons with abnormal pulse rates will be judged differently by the two methods, and in certain instances the latter might be a better test of normality. The final decision between them need not yet be made. The figures given in Table V define the normal range of the *work per minute-heart size* relationship in our data and permit the placing of individual results with regard to it.

SUMMARY

Duplicate estimations of cardiac output together with determinations of metabolism, blood pressure and pulse rate have been performed on 31 healthy persons and 204 hospital patients under conditions of basal metabolism. Orthodiagrams were secured also. The results have been subjected to statistical analysis.

The cases studied included patients with diseases not effecting the circulation, with hypertension, anemia, hyperthyroidism, neurocirculatory asthenia, valvular heart disease, various types of arrhythmia, coronary disease, acute endocarditis and aneurysm; also patients who had recovered from congestive heart failure. Acute cardiac decompensation, advanced pulmonary disease, and the febrile diseases were not studied.

The condition of the circulation in the various forms of disease has been described and compared with the normal. The most unexpected finding was that the average basal circulation in cases of neurocirculatory asthenia was very abnormal.

We have sought for relationships by which the condition of the heart muscle might be ascertained. Among normal persons, and patients with normal hearts but abnormal circulations, the relationship between heart work per beat and heart size holds more closely than any other studied. In cases who have been once decompensated this relationship is abnormal almost without exception. Therefore, we believe that it may be used to define normal myocardial function and to detect myocardial disease. Charts and equations are submitted by which the normality of any case can be decided.

TABLE VI

Original data and diagnoses of individual cases

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output		VII Pulse rate		VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
		years	*inches*	*pounds*	*liters per minute*		*per minute*		*mm. Hg.*	*cc. per minute*	*sq. cm.*	

Healthy Group.   Also cases 37, 43, 44, 45, 46, 47, 48

I	II	III	IV	V	VI		VII		VIII	IX	X	
51	F.	30	70	150	3.0	2.9	75	75	129–93	205	109	
52	M.	25	63	120	4.2	4.3	70	70	120–70	221	92	
53	M.	43	69	156	3.7	3.9	74	74	118–88	214	102	
67	M.	21	69	138	4.3	3.2	70	70	125–81	247	96	
97	M.	51	67	155	6.7	5.5	56	56	131–83	180	107	
103	F.	27	68	140	4.5	5.2	56	56	120–70		106	
107	M.	22	63	110	4.0	3.7	60	60	110–62	179	88	
120	M.	28	73	174	5.2	5.6	74	72	118–78	260	132	
122	F.	20	70	155	3.5	3.5	60	68	110–78	299	111	
126	M.	29	69	168	7.0	6.6	58	52	102–70	199	116	Athlete in training
152	M.	27	66	133	4.5	5.1	76	80	111–72	223	102	Sinus arrhythmia
198	F.	15	57	79	3.3	3.1	85	84	90–64	181	83	
220	M.	48	65	143	3.3	3.8	66	66	95–64	221	115	
223	M.	29	66	138	3.4		76	76	105–70	248	91	
224	M.	43	66	144	4.5	4.3	50	53	86–62	169	116	
225	M.	33	70	115	3.7	3.9	52	50	105–75	206	115	
227	F.	34	62	120	3.2	3.4	74	71	110–85	189	82	
228	M.	37	68	140	4.6		76	76	110–95	241	92	
229	F.	25	59	110	4.4	4.1	90	93	96–68	184	82	
230	M.	21	70	155	4.0	4.3	64	67	103–76	253	113	
231	M.	23	68	175	6.2	6.1	62	60	92–72	246	101	
232	M.	24	72	187	5.3	5.6	63	67	108–86	258	147	Athlete, but not in training
233	F.	46	62	115	2.9	2.8	60	61	104–73	174	84	
235	F.	22	64	125	2.3	2.2	79	79	102–78	163	78	

Hospital Patients with Normal Circulation.   Also cases 38, 39, 40, 41, 42

I	II	III	IV	V	VI		VII		VIII	IX	X	
70	M.	42	67	156	3.3	2.9	71	71	100–76	149	96	Duodenal ulcer
71	M.	41	67	146	3.2	2.6	65	65	120–85	189	94	Acute gastro-enteritis
72	F.	36	62	124	4.3	3.9	90	90	124–72	187	107	Diabetes mel. periph. vasc. disease
73	M.	54	64	135	3.1	2.5	64	60	106–69	233	104	Bronchial asthma, not in attack
77	M.	60	71	139	1.5	1.3	66	66	108–60	172	82	Gastric neurosis, weak and nervous
79	F.	30	65	142	5.4	3.6	84	74	115–65	199	101	Early pulm. tuberc.; myoma. uteri

TABLE VI (continued)

Hospital Patients with Normal Circulation. (continued)

I Case number	II Sex	III Age years	IV Height inches	V Weight pounds	VI Cardiac output liters per minute	VII Pulse rate per minute	VIII Average blood pressure mm. Hg.	IX O₂ consumed cc. per minute	X Cardiac silhouette area sq. cm.	Diagnoses and remarks
101	F.	76	60	166	3.8	76	108–82	209	140	Simple purpura; slight arteriosclerosis
110	M.	17	67	106	3.4	118	120–82	256	71	Psychoneurosis
115	F.	27	69	215	8.2	68	130–88	241	92	Obesity; simple goitre
123	M.	41	76	217	3.7	76	110–73	342	127	Very early Hodgkin's disease
121	M.	14	61	92	8.3	100	116–70	156	71	Lues III; enlarged inguinal glands
125	M.	31	67	130	7.0	72	116–76	205	116	Left rec. laryngeal paralysis; mediastinal tumor
129	M.	40	67	119	4.2	72	116–73	209	136	Chronic arthritis
132	M.	40	63	125	4.1	101	132–90	209	108	Diabetes mel.; lues III; malnutritional edema
133	M.	62	58	89	4.0	72	122–68	122	87	Diabetes mel.; moderate arterioscl.; very weak
136	F.	26	68	156	3.6	76	98–55	240	98	Chronic arthritis
137	M.	13	58	71	3.7	58	95–60	129	67	Former catarrhal jaundice
138	F.	30	60	129	4.8	98	133–80	199	85	Scoliosis; psychoneurosis
110	M.	27	60	98	3.3	72	107–69	160	76	Lues III
112	M.	55	68	137	1.9	54	105–65	226	130	Pyloric stenosis; duodenal ulcer; malnutritional edema
117	M.	15	69	131	3.1	68	126–69	221	87	Chronic nephrosis; edema
158	M.	28	69	160	4.5	72	126–85	270	110	Acute nephritis (?)
160	M.	59	65	136	4.7	56	108–65	212	98	Gastric neurosis; constipation
165	M.	43	66	150	2.9	49	101–60	209	101	Gallbladder disease (?)
166	M.	15	69	139	4.3	70	95–65	231	127	Convalescing from bronchopneumonia
168	M.	27	59	139	3.9	70	116–80	196	97	Optic atrophy; sinusitis
169	M.		66	128	4.5	68	110–66	245	127	Lues III; amputation below left knee
173	M.	22	56	71	3.0	61	85–55	187	71	Mediastinal tumor
176	M.	36	61	139	3.2	73	110–73	217	117	Lues III; convalescent from lobar pneumonia
177	M.	37	70	127	3.1	66	107–75	205	107	Thromboangiitis obliterans
181	M.	30	66	160	4.0	80	107–63	255	104	Pain in right side; nothing found
189	M.	25	66	138	2.7	69	108–76	252		Bronchiectasis (?); very little found
192	F.	11	58	116	1.7	67	122–80	220	88	Psychoneurosis; weakness
194	M.	28	63	88	2.6	61	91–70	160	65	Chronic ulcerative colitis
200	M.	35	63	118	3.3	100	91–66	230	98	No disease found
206	M.	65	69	175	4.1	80	108–60	230	119	Convalescent from lobar pneumonia
210	M.	41	67	157	1.1	61	116–68	239	109	Thromboangiitis obliterans
212	M.	35	68	111	3.8	60	122–73	211	100	Carcinoma of stomach (?)
217	F.	65	61	112	3.6	82	90–60	121	81	Tuberc. of skin and glands
226	M.	45	66	137	1.9	66	118–70	232	124	Carcinoma of colon?; weak, emaciated, senile
	M.	22	70	162	2.7	51	117–68	231	110	Inactive pulm. tuberc.
							109–80			Orthostatic albuminuria

								Remarks and diagnosis

Hypertensive Group. Also cases 1, 2, 3, 11, 3, and 13 inclusive.

							121	Diffuse toxic goitre
							98	Chronic glomerular nephritis; died 3 months later
							155	Chronic arteriolar nephritis
							110	Diffuse toxic goitre; on iodides
							85	Chronic arteriolar nephritis; on iodides
							170	Essential hypertension; died 1 month later
							110	Essential hypertension; mitral valvulitis?
							122	Essential hypertension; chronic arthritis; angina
							115	Chronic arteriolar nephritis; beginning uremia
							103	Brain tumor; died 1 month later
							23	Essential hypertension; obesity; died 1 month later
							156	Arteriosclerosis

Anemia Group. Also cases 19 and 20 *

							106	Carcinoma of stomach; Hb. 41%; R.B.C. 2.5
							98	Hemorrhage from duodenal ulcer; Hb. 70%; R.B.C. 4.0
							126	Bleeding duodenal ulcer; Hb. 37%; R.B.C. 2.0
							106	Primary pernicious anemia; very weak; Hb. 58%; R.R.C. 2.6
							91	Bleeding duodenal ulcer; Hb. 66%; R.B.C. 3.8

Hyperthyroid Group. Also cases 21, 22, 23*, 69*, and 89*

							121	Diffuse toxic goitre; B.M.R. +91%; on iodide
							91	Nodular toxic goitre; B.M.R. +46%; on iodide
							116	Diffuse toxic goitre; B.M.R. +29%; on iodide; conduction defect
							96	Diffuse toxic goitre; very sick; B.M.R. +60%; no iodide; Ekg. intraventricular
							104	Diffuse toxic goitre; B.M.R. +40%; on iodide
							90	Diffuse toxic goitre; B.M.R. +35%; on iodide; very sick
							112	Diffuse toxic goitre; B.M.R. +15% or higher; on iodide; diabetes mel.
							130	Diffuse toxic goitre; B.M.R. +65% or higher; on iodide; died post-operative,
							184	Nodular toxic goitre; B.M.R. +12.3% or higher; auricular fib.
							84	Diffuse toxic goitre; B.M.R. +49%; on iodide; old inactive tuberc.

TABLE VI (continued)

I Case number	II Sex	III Age (years)	IV Height (inches)	V Weight (pounds)	VI Cardiac output (liters per minute)	VII Pulse rate (per minute)	VIII Average blood pressure (mm. Hg.)	IX O₂ consumed (cc. per minute)	X Cardiac silhouette area (sq. cm.)	Diagnoses and remarks
					*Neurocirculatory Asthenia Group.* Also cases 33, 34, 35, 36					
76	F.	30	63	113	3.0	91	130–80	170	73	Ne. A. mucous colitis
92	F.	31	62	128	1.8	96	124–82	172	80	Ne. A.
128	M.	42	60	161	3.6	87	108–73	218	92	Ne. A.
139	F.	25	68	128	3.6	80	101–60	214	66	Ne. A.
111	F.	32	63	109	2.5	100	112–77	153		Ne. A.
116	M.	31	63	125	4.3	80	101–70		94	Ne. A.
162	M.	58	69	119	2.5	51	105–55	216	101	Ne. A. (?), pulm. tuberc.
201	F.	33	69	133	3.7	58	128–80	203	95	Ne. A.
222	F.	19	62	92	1.6	76	101–69	137	69	Ne. A.
					*Miscellaneous Group*					
51	M.	30	70	139	3.9	78	110–74	233	98	Multiple small arteriovenous anastomoses in left leg
91*	M.	21	65	115	2.5	62	98–66	218		Acute catarrhal jaundice
121*	M.	67	64	138	6.9	80	91–58	194		Extreme asthenia; cause unknown; mild diabetes mel.
131	M.	30	68	161	2.7	88	107–73	196	67	Addison's disease; bedfast, but improving
149	M.	20	70	110	5.5	62	112–73	235	110	Post-traumatic arteriovenous anastomosis on occiput
171	M.	43	62	152	5.3	81	115–65	216	114	Portal cirrhosis; ascites
189*	M.	21	64	91	1.7	56	90–68	141	82	Myxedema (?); pituitary cachexia (?); B.M.R. −28%
181	F.	41	59	93	2.6	81	97–60	168	82	Scleroderma; Addison's disease (?)
					*Hypotension.* Cases 37, 41, 70, 136, 137, 165, 189, 192, 198, 210, 220, 224, 229, 231. Found among preceding groups.					
					*Myocardial Disease Group.* Cases who have formerly had congestive heart failure. Also cases 24, 25, 26, 27, 28, 29. Found among preceding groups. The exercise tolerance was Class II B, or III					
57	F.	43	68	190	2.5	96	126–66	232	187	Luetic heart disease; aortic regurg.; Ekg; bundle branch block; twice in congestive failure; on digitalis; died 6 months later
58	F.	31	62	128	2.6	90	118–80	206	170	Rheum. heart disease; mitral, aortic and tricuspid valvulitis; aortic fib.; prolonged congestive failure; better, still has ascites; on digitalis; arterial oxygen normal
64	M.	48	67	130	2.7	93	215–150	262	182	Hypertensive heart disease (?); congestive failure 6 months ago; on digitalis
81	M.	39	67	133	2.6	72	107–71	205	191	Rheum. heart disease; mitral stenosis; just recovered from congestive failure; on digitalis

TABLE VI (*continued*)

Hypertension Group. Also cases 1, 3 to 11, 13 to 18 inclusive

I Case number	II Sex	III Age (years)	IV Height (inches)	V Weight (pounds)	VI Cardiac output (liters per minute)	VII Pulse rate (per minute)	VIII Average blood pressure (mm. Hg.)	IX O₂ consumed (cc. per minute)	X Cardiac silhouette area (sq. cm.)	Diagnoses and remarks
69	F.	32	68	148	5.4	100	170-90	308	121	Diffuse toxic goitre
75	F.	30	66	174	4.2	82	264-180	220	98	Chronic glomerular nephritis; died 3 months later
85	F.	41	65	180	3.1	50	161-110	162	154	Chronic arteriolar nephritis
89	F.	47	60	134	3.2	92	209-95	230	116	Diffuse toxic goitre; on iodides
109	M.	63	71	85	5.9	84	185-98	171	85	Chronic arteriolar nephritis; died 1 month later
117	F.	49	59	93	5.2	92	250-137	116	179	Essential hypertension; mitral valvulitis?
130	M.	66	68	153			163-80	199	110	Essential hypertension; chronic arthritis; argyria
159	F.	53	66	133	4.6	84	228-130	228	122	Chronic arteriolar nephritis; beginning uremia
164	F.	51	64	265	3.9	72	180-120	266	115	Essential hypertension; obesity; died 1 month later
193	F.	46	64	184	3.3	88	165-95	243	108	Brain tumor; died 1 month later
207	F.	53	65	159	2.7	72	150-107	155	93	Essential hypertension
213	M.	57	66	181	4.4	54	180-100	239	156	Arteriosclerosis

*Anemia Group. Also cases 19 and 20 **

I Case number	II Sex	III Age (years)	IV Height (inches)	V Weight (pounds)	VI Cardiac output (liters per minute)	VII Pulse rate (per minute)	VIII Average blood pressure (mm. Hg.)	IX O₂ consumed (cc. per minute)	X Cardiac silhouette area (sq. cm.)	Diagnoses and remarks
150	M.	73	65	128	3.1	68	112-75	148	106	Carcinoma of stomach; Hb. 41%; R.B.C. 2.5
175	M.	33	65	138	2.6	64	105-61	172	98	Hemorrhage from duodenal ulcer; Hb. 70%; R.B.C. 4.0
196	M.	43	66	140	6.5	84	90-52	190	126	Bleeding duodenal ulcer; Hb. 37%, R.B.C. 2.0
199	F.	59	58	144	5.3	98	160-85	169	106	Primary pernicious anemia; very weak; Hb. 58%; R.B.C. 2.6
211	F.	56	60	135	4.0	72	135-85	179	91	Bleeding duodenal ulcer; Hb. 66%; R.B.C. 3.8

Hyperthyroid Group. Also cases 21, 22, 23, 69*, and 89**

I Case number	II Sex	III Age (years)	IV Height (inches)	V Weight (pounds)	VI Cardiac output (liters per minute)	VII Pulse rate (per minute)	VIII Average blood pressure (mm. Hg.)	IX O₂ consumed (cc. per minute)	X Cardiac silhouette area (sq. cm.)	Diagnoses and remarks
74	F.	45	56	114	6.7	120	140-60	324	121	Diffuse toxic goitre; B.M.R. +91%; on iodide
80	F.	63	59	120	3.5	124	152-70	250	91	Nodular toxic goitre; B.M.R. +46%; on iodide
81	F.	55	65	102	4.4	78	129-85	222	116	Diffuse toxic goitre; B.M.R. +29%; on iodide; Ekg. intraventricular conduction defect
87*	F.	33	61	95	3.3	114	110-55	273	96	Diffuse toxic goitre; very sick; B.M.R. +60%; no iodide
106*	F.	42	62	106	2.6	90	132-74	248	104	Diffuse toxic goitre; B.M.R. +40%; on iodide; very sick
116	M.	40	66	123	3.7	108	128-72	302	90	Diffuse toxic goitre; B.M.R. +35%
191	F.	43	70	136	5.6	92	134-84	266	112	Diffuse toxic goitre; B.M.R. +15% or higher; on iodide; diabetes mel.
202	F.	52	65	174	5.1	108	108-71	365	130	Diffuse toxic goitre; B.M.R. +65%; on iodide; died post-operative
208*	F.	47	61	192	4.3	65	182-92	252	184	Nodular toxic goitre; B.M.R. +12.3% or higher; auricular fib.
214	F.	56	61	101	6.8	104	116-63	247	84	Diffuse toxic goitre; B.M.R. +49%; on iodide; old inactive tuberc.

TABLE VI (continued)

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output	VII Pulse rate	VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
		years	inches	pounds	liters per minute	per minute	mm. Hg.	cc. per minute	sq. cm.	
										Neurocirculatory Asthenia Group. Also cases 33, 34, 35, 36
76	F.	30	63	113	3.0 2.3	91 84	130–80	170	73	Nc. A. mucous colitis
92	F.	31	62	128	1.9 1.8	96 92	121–82	172	80	Nc. A.
128	M.	42	60	161	3.9 3.6	94 94	108–73	218	92	Nc. A.
139	F.	25	63	128	3.4 3.6	80 80	101–60	214	66	Nc. A.
111	F.	32	63	109	2.1 2.5	101 100	112–77	153		Nc. A.
146	F.	31	60	125	4.3		101–70		94	Nc. A.
162	M.	58	60	119	2.3 2.5	61 61	105–55	216	101	Nc. A. (?), pulm. tuberc.
201	M.	33	62	133	3.3 3.7	54 58	128–80	203	95	Nc. A.
222	F.	19	62	92	1.4 1.6	76 76	101–69	137	69	Nc. A.
										Miscellaneous Group
51•	M.	30	70	139	3.9 2.3	78	110–74	233	98	Multiple small arteriovenous anastomoses in left leg
91•	F.	21	65	115	2.5 6.0	62	98–66	218		Acute catarrhal jaundice
121•	M.	67	68	138	6.0 2.7	80	91–58	194		Extreme asthenia; cause unknown; mild diabetes mel.
149	M.	30	70	161	2.3 5.5	88	107–73	196	67	Addison's disease; bedfast, but improving
149	M.	20	70	140	5.1 5.3	62	112–73	235	110	Post-traumatic arteriovenous anastomosis on occiput
171	M.	43	62	152	5.5 1.7	81	115–65	216	114	Portal cirrhosis; ascites
180•	M.	21	61	91	1.6 1.2	56	90–68	141	82	Myxedema (?); pituitary cachexia (?); B.M.R. − 28%
181	F.	41	59	93	2.2	80	97–60	168	82	Sclerolemn; Addison's disease (?)
										Hypotension. Cases 37, 41, 70, 136, 137, 165, 189, 192, 198, 210, 220, 224, 229, 231. Found among preceding groups
										Myocardial Disease Group. Cases who have formerly had congestive heart failure. Also cases 24, 25, 26, 27, 28, 29. The exercise tolerance was Class II B, or III
57	F.	43	68	190	2.5 2.5	96 96	126–66	232	187	Luetic heart disease; aortic regurg.; Ekg; bundle branch block; twice in congestive failure; on digitalis; died 6 months later
58	F.	31	62	128	2.6	90	118–80	206	170	Rheum. heart disease; mitral, aortic and tricuspid valvulitis; nuric. fib.; prolonged congestive failure; better, still has ascites; on digitalis; arterial oxygen normal
63	M.	18	67	130	2.8 2.7	93 93	215–150	262	182	Hypertensive heart disease (?); congestive failure 6 months ago; on digitalis
81	M.	39	67	133	3.5 2.6	72 72	107–71	205	191	Rheum. heart disease; mitral stenosis; just recovered from congestive failure; on digitalis

TABLE VI (continued)

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output	VII Pulse rate	VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
		years	inches	pounds	liters per minute	per minute	mm. Hg.	cc. per minute	sq. cm.	

Myocardial Disease Group. (continued)

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output	VII Pulse rate	VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
90	M.	64	70	169	2.7 / 2.7	72 / 70	122–76	210	114	Gout; angina pectoris; year ago, 2 attacks of auric. fib. with rapid rate and congestive failure; recovered when rhythm returned to normal; now normal rhythm
118	F.	45	61	163	5.2 / 3.1	72 / 64	116–79	201	160	Rheum. heart disease; mitral stenosis; chronic arthritis; congestive failure 6 months before
145	M.	72	67	170	3.7 / 2.5	80 / 80	160–70	125	235	Arterioscl. heart disease; auric. fib.; just recovered from first congestive failure; on digitalis
151	M.	63	67	155	2.5 / 2.6	62 / 58	112–72	216	138	Arterioscl. heart disease; auric. fib.; just recovered from first congestive failure; on digitalis
153	F.	48	61	188	3.5 / 3.5	96 / 96	120–75	223	144	Rheum. heart disease; auric. fib.; just out of first slight failure; on digitalis
156	F.	50	61	129	2.6 / 2.9	68 / 64	195–124	271	176	Rheum. heart disease (?); auric. fib.; first congestive failure 8 months ago; on digitalis
167	F.	48	60	122	2.8 / 3.0	108 / 92	165–110	207	185	Rheum. heart disease; mitral and aortic valvulitis; auric. fib.; just out of first mild failure; on digitalis
172	M.	38	65	205	3.9 / 3.9	88 / 88	204–140	298	207	Hypertensive heart disease; apparently in congestive failure 1 year before; ascites now; cardiac cirrhosis; on digitalis
195	F.	49	69	136	2.8 / 2.6	46 / 46	128–80	168	162	Rheum. heart disease; mitral stenosis; in slight failure 3 months ago; too much digitalis now
209	F.	67	62	140	3.6	80	133–72	191	148	Probably rheum. heart disease; in failure 1 month ago; attacks of paroxysmal cardiac dyspnea and auric. fib.; now normal rhythm

Valvular Heart Disease Group–Regular Rhythm. Never in congestive failure. Also case 49.

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output	VII Pulse rate	VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
100	F.	21	60	121	3.2 / 3.7	62 / 60	115–86	210	181	Rheum. heart disease; mitral stenosis; tricuspid stenosis (?). Class II B
102	F.	20	66	120	3.7 / 3.9	72 / 64	107–82	239	83	Rheum. heart disease; mitral stenosis. Class I
135	M.	27	68	128	7.5 / 6.6	80 / 68	158–69	314	149	Rheum. heart disease; mitral and aortic valvulitis. Class II A
184	M.	23	73	168	4.2 / 3.7	76 / 72	123–65	281	185	Rheum. heart disease; aortic regurg. predominates. Class II A
185	M.	48	63	164	5.4 / 5.0	88 / 82	183–72	211	161	Arterioscl. heart disease; aortic regurg. Class II A
187	M.	60	66	112	2.0 / 1.9	80 / 84	164–80	229	121	Lues III; slight aortic regurg. Class II A
201	M.	56	66	157	3.7 / 4.3	76 / 72	140–45		153	Lues III; aortic regurg. Class II A

TABLE VI (continued)

I Case number	II Sex	III Age	IV Height	V Weight	VI Cardiac output	VII Pulse rate	VIII Average blood pressure	IX O₂ consumed	X Cardiac silhouette area	Diagnoses and remarks
		years	inches	pounds	litres per minute	per minute	mm. Hg.	cc. per minute	sq. cm.	
Arrhythmia Group A. Arrhythmic when tested. Never in congestive failure. Also case 50.										
55	F.	43	60	108	1.6	160	120–90	204		Rheum. heart disease; digitalis. Class II B
56	M.	58	66	113	2.3	161	100–80	238	120	Paroxysmal tachycardia, auricular type; arterioscl. Class II A
105	F.	47	59	153	4.3	102	180–110	247	132	Hypertensive heart disease; auric. fib.; coronary occlusion (?); on digitalis. Class III
205	M.	54	71	170	3.3	74	110–75	192	107	Auric. fib.; cause unknown; no digitalis. Class II A
208	F.	47	61	192	4.1	66	182–92	252	184	Auric. fib.; adenoma of thyroid; not thyrotoxic now; on digitalis. Class II B
219	M.	54	66	154	2.0	28	175–66	131	161	Complete heart block for 5 years; rheum. heart disease (?). Class I
221	M.	42	60	191	4.6	186	90–70	302	92	Paroxysmal auric. tachycardia. Class II B
214	M.	71	66	125	2.5	26	170–62	93		Complete heart block; arterioscl. Class III
Arrhythmia Group B. In normal rhythm when tested. Never in congestive failure. Also case 50.										
55	F.				2.3	88	130–90	243	174	Paroxysmal auric. fib. See above
56	M.				3.3	76	122–80	295	1.12	Paroxysmal tachycardia. See above
80, 81, 116		60		134	3.2 / 4.3	92 / 90				History of attacks of cardiac irregularity; type unknown. See under Thyroid Group
89	F.				2.8	96	200–95 95–75	230	110	Diffuse toxic goitre; former attacks of auric. fib.; on iodide
221	M.	47				72 / 72		261	1.30	Paroxysmal auric. tachycardia. See above

TABLE VI (continued)

I Case number	II Sex	III Age (years)	IV Height (inches)	V Weight (pounds)	VI Cardiac output (liters per minute)	VII Pulse rate (per minute)	VIII Average blood pressure (mm. Hg.)	IX O₂ consumed (cc. per minute)	X Cardiac silhouette area (sq. cm.)	Diagnoses and remarks

O₂ = O_2

Coronary Group. None ever in congestive failure. Also cases 2, 31

I	II	III	IV	V	VI	VII	VIII	IX	X	Diagnoses and remarks
88	M.	63	68	160	5.9 / 4.1	74 / 74	220–94	204		Diabetes mel.; coronary occlusion 3 months before (?); died 3 weeks later; necropsy showed old and fresh infarcts
98	F.	52	62	143	2.6 / 3.6	64 / 64	138–77	159	104	Diabetes mel.; coronary occlusion 2 months before
105	F.	47	59	153	4.4	102	180–110	247	132	Auric fib.; coronary occlusion 8 days before
108	M.	68	67	175	9.9 / 8.9	83 / 86	104–70	236		Coronary occlusion month before; died next day; necropsy showed old and fresh infarcts
119	M.	63	67	135	2.2 / 1.1	58 / 57	96–60	202	136	Lues; abdominal aneurysm; angina pectoris
144	M.	47	65	184	3.9 / 4.5	104 / 104	104–80	204	145	Coronary occlusion 2 months before
163	M.	50	63	146	5.3 / 4.3	88 / 88	131–78	210	125	Coronary occlusion 1¼ months before; angina pectoris previously
197	F.	58	61	123	2.9 / 3.2	68 / 70	152–80	192	108	Diabetes mel.; coronary occlusion 3 weeks before

Acute Endocarditis

I	II	III	IV	V	VI	VII	VIII	IX	X	Diagnoses and remarks
135	M.	27	67	137	4.5 / 3.4	88 / 64	160–55	224	141	Rheum. heart disease; mitral and aortic valvulitis; active (?)
186	M.	22	68	118	3.0	84	97–60	200	109	Congenital heart disease; pulmonary stenosis; subacute bacterial endocarditis
188	F.	15	49	98	2.5 / 3.0	73 / 72	104–65	176	80	Acute rheum. fever with cardiac involvement
190	M.	28	67	158	3.7 / 4.6	68 / 68	110–49	267	125	Acute rheum. fever with cardiac involvement
215	F.	30	67	168	5.8 / 5.3	92 / 100	135–88	213	103	Acute rheum. fever, 2d attack; cardiac involvement

Aneurysms. Also case 119

I	II	III	IV	V	VI	VII	VIII	IX	X	Diagnoses and remarks
82	M.	42	70	134	2.7 / 3.5	82 / 83	190–72	196	132	Lues; aneurysm of ascending aorta
93	M.	48	55	174	5.2 / 4.3	64 / 58	159–40	202	168	Lues; aneurysm of aortic arch
113	M.	45	70	137	3.4 / 3.2	74 / 74	110–52	226	144	Lues; aneurysm of aortic arch
114	M.	49	64	111	2.5 / 2.7	88 / 84	130–82	200	102	Lues; aneurysm of ascending aorta

* Cases omitted from the statistical analysis because of doubt, from clinical criteria, concerning the condition of their hearts.

TABLE VII

Supplementary data of cases published previously *

Case number	Sex	Age	Height	Weight	Case number	Sex	Age	Height	Weight	Case number	Sex	Age	Height	Weight
		years	inches	pounds			years	inches	pounds			years	inches	pounds
1	M.	62	73	168	9	F.	54	54	138	17	F.	76	69	140
2	M.	63	73	170	10	M.	57	73	189	18	M.	30	72	180
3	M.	58	66	146	11	F.	46	57	103	19	M.	37	66	155
4	F.	60	61	134	12	M.	67	69	175	20	M.	47	65	147
5	F.	44	62	235	13	F.	40	62	133	21	F.	20	61	105
6	F.	60	64	144	14	F.	53	61	168	22	F.	30	69	119
7	M.	50	65	142	15	M.	23	72	131	23	F.	22	62	117
8	M.	55	65	130	16	F.	54	65	159	24	M.	24	65	115
25	M.	41	68	107	34	M.	34	67	154	43	M.	34	72	180
26	M.	49	60	130	35	F.	24	61	108	44	M.	26	73	160
27	M.	45	65	143	36	M.	23	60	114	45	M.	36	72	166
28	F.	52	62	112	37	F.	18	63	106	46	M.	28	71	165
29	M.	46	70	179	38	M.	27	70	123	47	M.	26	67	132
30	M.	45	66	145	39	M.	26	70	143	48	M.	36	67	143
31	M.	50	62	107	40	M.	23	73	157	49	F.	33	70	160
32	F.	20	59	135	41	M.	21	68	137	50	M.	26	64	151
33	M.	45	60	124	42	M.	50	66	148					

* The sex, age, height and weight only are given. Diagnoses and other findings are on Table IV of a previous paper (1).

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THE RADIATION OF HEAT FROM THE HUMAN BODY

I. An Instrument for Measuring the Radiation
and Surface Temperature of the Skin

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In the past few years the interest in an accurate knowledge of the temperature of the surface of the skin and of the energy radiated therefrom has greatly increased. This interest has been due on the one hand to the important part played by radiation in the theories of ventilation and on the other to the desire to make a more accurate analysis of the factors involved in human metabolism. Human calorimetry has developed to the stage where it is possible to determine accurately the total amount of heat eliminated from the body, and also, to some extent, to separate factors contributing to this total. It is generally recognized that the human body eliminates practically all of its energy from its surface in three ways, namely, by conduction and convection, evaporation of moisture, and radiation. These factors are variable depending upon the condition of the individual and upon the surrounding environment. Aside from the losses due to vaporization, the energy from the body, liberated by radiation and conduction, probably follows Newton's cooling law and for that reason the temperature of the surface of the body is of physiological importance. Therefore in order that a possible relation between the metabolic rate and surface temperature and radiation may be investigated it is necessary that experiments be carried out with an instrument which will measure these latter quantities accurately and in absolute value. It is believed, for reasons to be discussed in the next paper (1), that a radiometric device, which depends upon the absorption of the radiant energy of the skin for its indications, is the simplest and most accurate instrument for the measurement of skin temperature and radiation. The present design of instrument is described with its technique because it offers several advantages over previous designs so far as accuracy and general simplicity are concerned.

The use of a radiometer [1] for the measurement of radiation and skin temperature is now some twelve years old, having been first employed by

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[1] The term " radiometer " is employed in this paper to refer to any device for measuring radiation and not alone to the physical instrument generally known by that name—the physical instrument of Crookes.

Aldrich (2, 3) in 1922 and later by Cobet and Bramigk (4) and by Boh-nenkamp and Ernst (5).

These instruments all require the use of a very sensitive galvanometer and thus they are not suitable for general use. The devices of Aldrich and of Cobet and Bramigk are adapted to the measurement of skin temperature and that of Bohnenkamp and Ernst to measurement of body radiation. Recognizing the desirability of using a radiometer for general measure-ments of this kind the present apparatus, measuring at the same time skin temperature and body radiation, was designed to be rugged, simple, and accurate.

The apparatus as assembled for use on a rolling table is shown in the photograph, Figure 1. An idea of the size of the device can be had from the upper scale of the slide rule, S, which is marked off in centimeters; the whole slide rule is 12 inches long. The apparatus consists essentially of three parts, the radiometer, R, the galvanometer, Ga, and the potentiom-eter, P.

The galvanometer is a product of Leeds and Northrup and is listed in their catalogue as Number 2400, with a sensitivity of 10 micro-volts per division. Instead of the usual telescope, a light has been mounted in place of the eyepiece and an index is projected upon the scale, GS, so that the galvanometer deflection is much easier to observe.

The potentiometer is of my own design and was built in this laboratory by Mr. G. F. Soderstrom. A wiring diagram of the circuit is shown in Figure 2. The current through the one ohm coil at 'output' is deter-mined by the current through the milliammeter and the resistances v or v'. When the switch S_1 is thrown to put v or v' in the circuit the voltage across the output coil is 10^{-4} or 10^{-5} volts respectively. The switch S throws the variable rheostat R in parallel with either v or v' so that any arbitrary volt-age can be produced across the output coil per milliampere, depending upon the setting of the rheostat. The rheostat is so set upon calibration that the milliammeter reads one millampere for each small calorie of radiant energy $\times 10^{-4}$, per second per cm². The resistance coils for the potentiometer are wound of constantan wire so that no trouble is encountered while using the instrument within the usual range of temperatures. The flexible leads to the radiometer are marked $F.R.$

The radiometer itself consists of two parts shown in Figure 1, the radiation sensitive device, R, and the room temperature black-body, $R.T.B.$, or cold body. This latter is an aluminum block which is painted black and which serves as a reference body for the radiometer. Its temperature is determined by means of a right-angle mercury thermometer, T, attached to the block. The temperature of this block is not kept constant but changes with the room temperature and unless some large, rapid fluctuation occurs it can be considered as at the temperature of the room.

In Figure 3 is shown a vertical section of the radiation device together
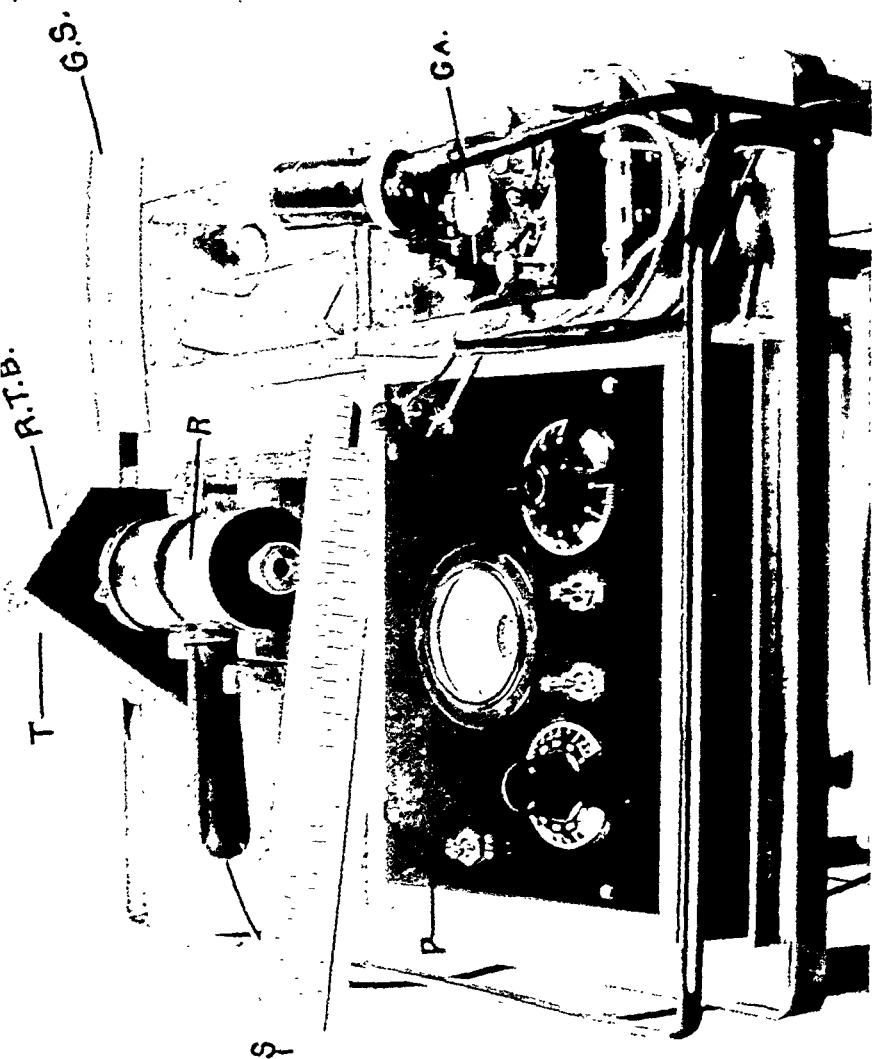
FIG. 1. RADIOMETRIC SURFACE THERMOMETER ASSEMBLED FOR USE

with the calibration set, or Leslie Cube. The outside casing of the radiometer is a cylindrical brass tube, one end of which is closed with a metal cap and the other end partially closed by a metal ring into which are screwed four metal feet provided with amber tips. The inside casing is a fiber tubing which is separated from the metal tube by a ring of cork. This

FIG. 2. WIRING ARRANGEMENT FOR POTENTIOMETER

fiber tube is closed at one end by a fiber block which serves as the support for the thermocouples, T. Directly in front of the thermocouples is placed a thick fiber block in the center of which is fitted a silver cone, C, for concentrating the rays upon the thermocouples. There is no shutter or foreign medium between the thermocouples and the skin. In the upper left corner of the same figure is shown a detailed diagram of the thermocouple arrangement in plan. The stippled rings are the fiber supports over which is stretched a thin (1 mu) film of nitrocellulose, N. This film gives added support to the thermocouples without changing the radiation characteristics of the thermopile. The radiation thermopile consists of four junctions arranged in the form of a disc as shown at T. The disc is of tinfoil and is divided into four parts so that each junction is insulated from the other three. The cold junctions, J, are arranged symmetrically about the radiation junctions outside the range of the incident radiation. The elements of the thermocouples are the Hutchins bismuth alloys (95 per cent Bi—5 per cent Sn and 97 per cent Bi—3 per cent Sb) and are made by myself according to a method devised by Professor A. H. Pfund of Johns Hopkins University, Baltimore. (Professor Pfund informs me that he is shortly to publish a full account of his new technique for thermopile construction.) The elements are welded together mechanically and the tin foil receivers welded to the junction of the two elements. The thermoelectric power of the thermocouples is approximately 130 microvolts per degree, and this combination of metals is now used quite extensively in sensitive radiation

devices.   The cold junctions serve in a compensatory character and, while not affecting the radiation readings, help in maintaining a constant zero position in the galvanometer and minimize the effect of stray disturbances. The metal disc has a diameter of 9 mm. and its surface is blackened by depositing bismuth-black in a vacuum.   This metal black is totally absorbing for all wave lengths in the visible and near infra red but becomes more reflecting as the wave length increases, so that at 10 mu, where the maximum of body radiation occurs, it absorbs only about 60 per cent of the incident energy.   Several other blackening materials were experimented with but none were found to be as satisfactory as bismuth-black.   The introduction of the silver cone in front of the thermopile increases its sensitivity about ten times so that the instrument can be used with the relatively insensitive portable galvanometer.   The instrument can be handled freely and air currents, etc. have little effect upon it.
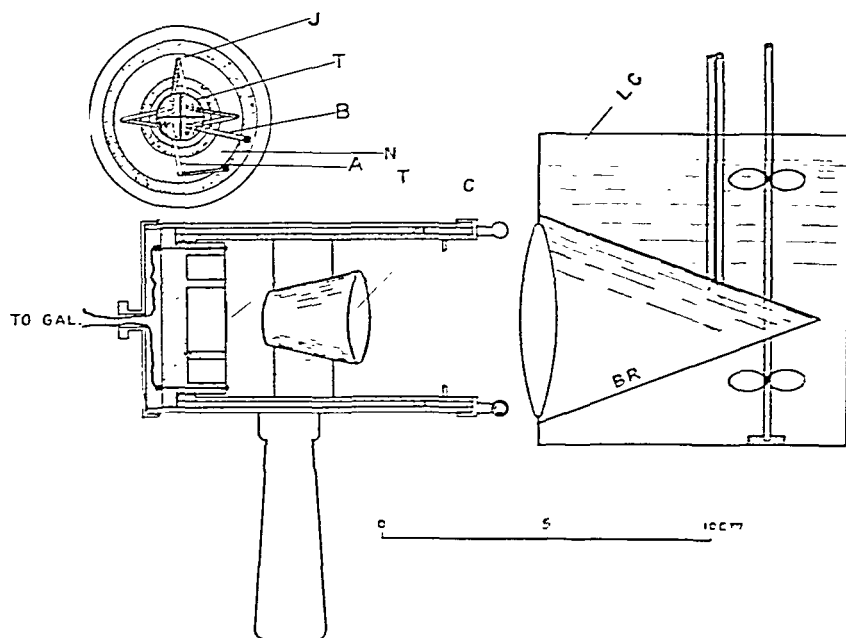
FIG. 3.   VERTICAL SECTION OF RADIOMETER (LOWER LEFT) AND LESLIE CUBE (RIGHT).   DETAIL OF THERMOPILE CONSTRUCTION (UPPER LEFT)

The Leslie Cube, LC, is shown to the right of the radiometer in Figure 3.   This radiation standard consists of a metal cube 10 cm. on an edge with a hole 7 cm. in diameter in one side over which is fitted a copper cone, BR, extending into the cube.   The cone is painted black with a glossy paint so that one looks into the base of the cone as if looking into a deep hole.   The cube is provided with a stirrer and thermometer, and for a calibration is

with the calibration set, or Leslie Cube. The outside casing of the radiometer is a cylindrical brass tube, one end of which is closed with a metal cap and the other end partially closed by a metal ring into which are screwed four metal feet provided with amber tips. The inside casing is a fiber tubing which is separated from the metal tube by a ring of cork. This

FIG. 2. WIRING ARRANGEMENT FOR POTENTIOMETER

fiber tube is closed at one end by a fiber block which serves as the support for the thermocouples, T. Directly in front of the thermocouples is placed a thick fiber block in the center of which is fitted a silver cone, C, for concentrating the rays upon the thermocouples. There is no shutter or foreign medium between the thermocouples and the skin. In the upper left corner of the same figure is shown a detailed diagram of the thermocouple arrangement in plan. The stippled rings are the fiber supports over which is stretched a thin (1 mu) film of nitrocellulose, N. This film gives added support to the thermocouples without changing the radiation characteristics of the thermopile. The radiation thermopile consists of four junctions arranged in the form of a disc as shown at T. The disc is of tinfoil and is divided into four parts so that each junction is insulated from the other three. The cold junctions, J, are arranged symmetrically about the radiation junctions outside the range of the incident radiation. The elements of the thermocouples are the Hutchins bismuth alloys (95 per cent Bi—5 per cent Sn and 97 per cent Bi—3 per cent Sb) and are made by myself according to a method devised by Professor A. H. Pfund of Johns Hopkins University, Baltimore. (Professor Pfund informs me that he is shortly to publish a full account of his new technique for thermopile construction.) The elements are welded together mechanically and the tin foil receivers welded to the junction of the two elements. The thermoelectric power of the thermocouples is approximately 130 microvolts per degree, and this combination of metals is now used quite extensively in sensitive radiation

emitted by the cone, the variable rheostat is adjusted until the two coincide. The calibration is then finished and as many points as desired can be taken to check the setting of the rheostat. For the present design of radiometer, a radiation of 10^{-3} small calories per second per cm^2 from the cone will produce 150 mm. deflection upon the galvanometer, corresponding to a voltage of 2×10^{-4} volts. A calibration usually takes about one minute so that a calibration check is carried out before each day's work. The setting of the rheostat can be trusted for several weeks.

The potentiometer is employed in order to make the results independent of the galvanometer, which is used only as a null instrument. However, if readings are to be made within a small range of room temperatures the galvanometer can be trusted as a deflection instrument. In this case, a galvanometer scale is drawn so that the deflection of the galvanometer can be read directly in calories or in temperature. This method is not quite so precise as that with the potentiometer, but, with sufficient care, temperature can be read in this manner with an accuracy of about $\pm 0.2^\circ$ C.

The indications of the galvanometer and of the potentiometer are proportional to the energy exchange between the cube and the $R.T.B.$, and therefore the readings are usually made in terms of energy and converted by the ' slide rule ' into temperature. The temperature difference between the $R.T.B.$ and the cube is not proportional to the deflection of the galvanometer except for very small temperature differences. This is to be expected from the expansion of the Stefan-Boltzmann formula.

$$ S = S_o \ (4T_0^3 \ \overline{\Delta T} + 6T_0^2 \ \overline{\Delta T^2} + \overline{4T_o} \Delta T^3 + \overline{\Delta T^4}). $$

where $\Delta T = T - T_o$.

As long as ΔT is small compared to T_o, all the terms except the first may be neglected, but for ΔT as large as 10° C. the second degree terms are appreciable and errors as large as 0.5° C. may be made by neglecting the deviation of the temperature curve from a straight line Cobet and Bramigk (4) state that the temperature calibration curve for their radiometer was a straight line in contradiction to the above statements. Should their device have been as accurate as reported, $\pm 0.1^\circ$ C. it would seem impossible that they should not have detected the deviation mentioned, since, at $\Delta T = 10$, it amounts to 0.5° C. The fact that their instrument measures the temperature difference between the hot source and the movable shutter, whose temperature is not accurately known, probably accounts for the discrepancy. A shutter was built into the present radiometer in a manner very similar to that of Cobet and Bramigk, but it was found to introduce appreciable uncertainties into the results. To take the place of the shutter the $R.T.B.$ was designed so that the radiometer simply compares the radiation of the skin or Leslie Cube with that of the $R.T.B.$ whose temperature is known.

filled to a level above the cone with warm water. The temperature of the surface of the cone is assumed to be at the same temperature as that of the water in the cube. This assumption seems to be justified by the fact that the heat losses per unit area of the conical surface are small. Thus the cone serves two important purposes, that of being a very efficient black-body radiator and being so arranged that its temperature can be easily measured. Actual tests show that readings on the cone, whether the cube temperature be above or below room temperature, are perfectly consistent, confirming the assumption that the temperature of the conical surface is practically that of the water of the cube.

The technique of calibration is as follows: The radiometer is placed in its holder (see Figure 1) so that the thermocouples are facing the blackened surface of the $R.T.B.$ The temperature of the $R.T.B.$ is noted and the galvanometer index is set to zero. The switch of the potentiometer battery is open so that no e.m.f. is imposed across the "output" coil. The radiometer is then placed in the position indicated in Figure 3, and the temperature of the water in the cube noted. The deflection of the galvanometer is balanced off by means of the potentiometer, which has been adjusted so that the switch, S, has connected the variable rheostat in the circuit. The energy radiated from the cone per unit area of its base is computed from the Stefan-Boltzmann formula,

$$S = S_0 \ (T^4 - T_0^4) \tag{1}$$

where, S = radiation emitted in small calories per second per square centimeter of surface (on the surface base).

S_0 = Stefan-Boltzmann constant = 1.37×10^{-12} small cal/sec/cm².

T = the absolute temperature of the cube = $273 +$ deg. C..

T_0 = the absolute temperature of the $R.T.B.$

This calculation can be quickly done graphically by means of the slide rule which is shown at S in Figure 1. The rule is made up of two scales one proportional to the calories of heat radiated by the cube and the other proportional to the centigrade temperature of the cube as computed from the above formula. This mechanical monograph is a great time saver in the matter of calculation. Thus, by setting the cold body temperature on the zero of the energy scale, one reads off the energy that should be indicated by the potentiometer, by noting the energy which corresponds to the cube temperature. As an example, consider the setting of the slide rule shown in Figure 1. The upper scale is the energy or caloric scale and the lower the temperature scale. The zero of the energy scale is set for $R.T.B.$ temperature of 22° C. Let us suppose that the cube temperature is 34.5° C., then the milliameter of the potentiometer should read 18.75 milliamperes corresponding to a radiation of 18.75×10^{-1} small calories per second per cm². If the reading in milliamperes does not equal the number of calories

from the cube is restricted to a cone of rays and the energy received by the
thermocouples can be calculated by use of the Stefan-Boltzmann and Lam-
bert cosine laws.   The result is obtained by considering the transfer of
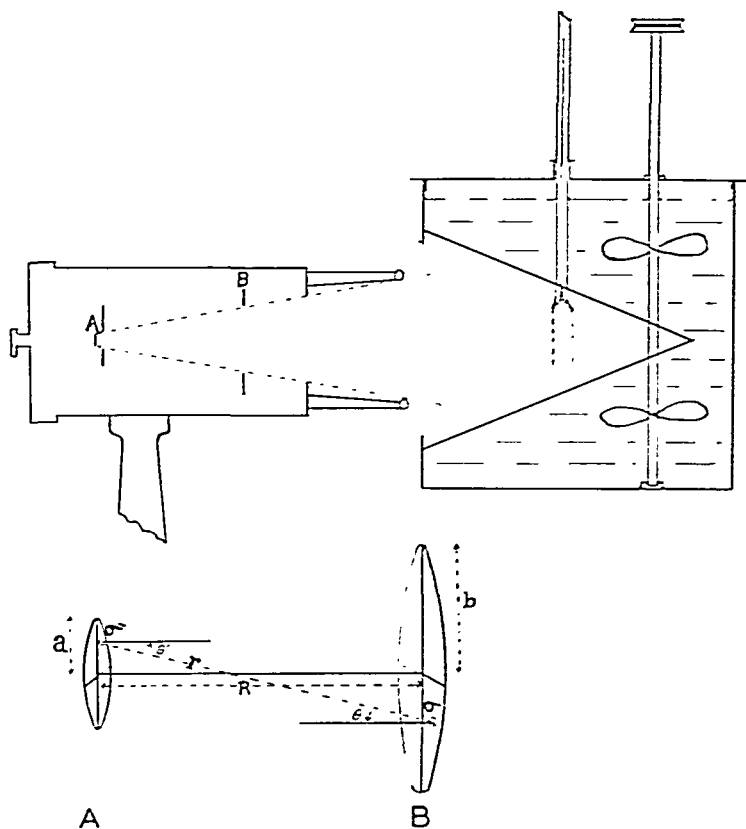
FIG. 5.   DIAGRAM FOR CALCULATION OF ENERGY RECEIVED BY
THERMOPILE FROM LESLIE CUBE

energy between the two plane, parallel, circular surfaces A and B. A being
the area of the thermocouple disc and B the area of the diaphragm.   Inte-
gration yields as the result.

$$Q = \frac{S}{\pi} \frac{AB}{R^2} \left(1 - \frac{a^2 - b^2}{R^2} \right).$$ (2)

where Q = interchange of energy between the Leslie Cube and the R.T.P.
S = defined in Equation 1.
R = distance of thermocouple from diaphragm.
a = radius of receiving surface A.
b = radius of emitting surface B.

In Figure 4 is shown a photographic registration of the galvanometer deflections. The baseline shows that the *R.T.B.* was at a temperature of 24.8° C. and the temperature of the cube is indicated by the ordinates along with the energy exchange. The abscissae indicate that about eighteen or twenty seconds is required for a reading. In fact, 98 per cent of the final value is reached in this time.
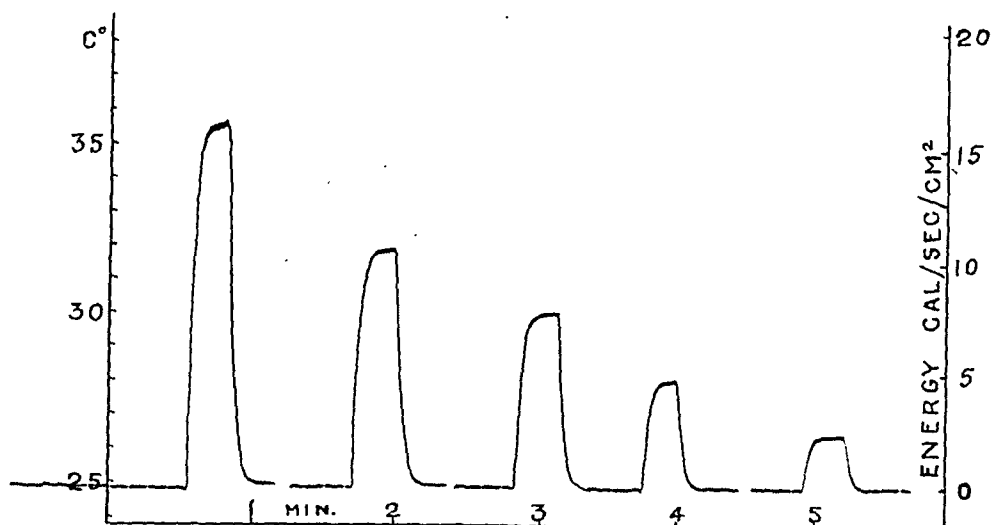
FIG. 4. PHOTOGRAPHIC REGISTRATION OF RADIOMETER CALIBRATION

The calibration of the instrument is easily accurate to ± 0.05° C. but the usual readings made upon subjects are accounted accurate to ± 0.1° C. and the energy readings to ± 0.1 × 10⁻⁴ small cal/sec/cm². The measurement which is made is the transfer of energy from the skin to a black-body at room temperature and in this process the radiometer plays only the rôle of a comparator and does not make any absolute measurement in itself. (The rise in temperature of the radiation junctions, when the instrument is in use does not invalidate this statement.) Without this technique, many instrumental constants, among them the absorbing power of the blackening material with which the radiation junctions are covered, would enter as a correcting factor. These complications of the data are avoided by making the radiometer play the part of a 'thermo-level' which compares the thermometric level of the skin with that of the R.T.B. whose temperature is known. This technique is similar to that used by Aldrich.

In order that the technique of the 'thermo-level' be checked as to absolute value of the radiation, tests were made using a radiation standard furnished by the U. S. Bureau of Standards. The comparison between the radiation standard and the Leslie Cube calibrations can be more easily made if the geometry of the radiometer be made as simple as possible. To this end the condensing cone in the radiometer was replaced by a diaphragm of appropriate size as shown at A in Figure 5. Then the radiation coming

sons, and they give the value of 1.18×10^{-9} cal/sec/cm^4 as being the average. Converting this into the form of S (small cal/sec/cm^2), Equation 1, this value becomes 3.17×10^{-2} small cal/sec/cm^2 corresponding to a skin temperature (160° C.) 320° F. The corresponding value obtained with the radiometer under similar circumstances is $S = 1.50 \times 10^{-3}$ small cal/sec/cm^2, skin temperature = (34.9° C.) 96° F. An examination of the data in the paper of Bohnenkamp and Ernst shows that their radiation values are on the average 17 times those obtained with the radiometer. The computations which lead from these data to their very reasonable result, that their subject lost 70 per cent of her basal heat through radiation, were gone over,[2] and it appears that a factor of approximately $2\pi^2$ (19.6) was lost in the calculation. Their errors, therefore, probably originate in their radiometric device. Their whole method of calculation of the projection surface area of the body is very fine, but their technique in the measurement of radiation is questionable and provides possibilities of errors of the magnitude mentioned above.

When the radiometer is placed before the warm skin or Leslie Cube for any length of time the inner structures of the instrument are warmed and on account of their re-radiation produce an additional deflection upon the galvanometer. Upon replacing the instrument before the cold $R.T.B.$ it is found that the index does not return quite to the zero position. This effect is not observed for temperature differences less than 5° C. or when the radiometer is referred after each observation to the $R.T.B.$ It is often desirable, however, to make a series of measurements without going to the trouble of referring the radiometer to the $R.T.B.$ after each reading. In order to do this the radiometer is first warmed up to the temperature level which is to be investigated, by allowing it to point at the skin of the subject for one minute before an observation is to be taken. Then the instrument is referred to the $R.T.B.$ the index of the galvanometer adjusted to zero, and the radiometer put into use immediately. Usually the whole surface of the body can be investigated in one series of readings with the exception of cold extremities. To examine the cold extremities the radiometer is replaced in front of the $R.T.B.$, the zero position checked, and the hands and feet examined as rapidly as possible. Tests have shown that no appreciable error is introduced by such a procedure. There is no danger of mistaking the slow rise in deflection due to the heating of the instrument with that due to the heating of the thermocouples, and a very definite equilibrium point is reached in each case.

There are several items of theory concerning the determination of skin temperature by means of a radiation device, such as the radiating power of

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[2] The calculations of Bohnenkamp and Ernst as well as my own were carefully checked by Dr. Grundfest of the Department of Physiology, Cornell Medical College and by Dr. K. Hartline of the Johnson Foundation, University of Pennsylvania Medical School. The author is greatly indebted to Dr. Grundfest and Dr. Hartline for their kindness.

The values of Q, obtained by changing the temperature of the Leslie Cube, are then plotted against the e.m.f. developed by the thermocouples, and compared with the curve obtained with radiation standard under the condition prescribed by the Bureau of Standards. Table I shows the results of a calibration comparison between the radiation standard and the Leslie Cube.

TABLE I

Calibration against standard lamp and Leslie Cube

	S	E.M.F.	S/E
	$cal/sec/cm^2$	*volts*	
Standard lamp.................	1.20×10^{-4}	32.6×10^{-7}	36.8
Standard lamp.................	1.75×10^{-4}	47.0×10^{-7}	37.2
Standard lamp.................	2.45×10^{-4}	67.0×10^{-7}	36.6
Standard lamp.................	3.20×10^{-4}	86.5×10^{-7}	36.9
Leslie Cube..................	3.70×10^{-4}	100×10^{-7}	37.0
Leslie Cube..................	10.2×10^{-4}	273×10^{-7}	37.4
Leslie Cube..................	15.5×10^{-4}	417×10^{-7}	37.2
Leslie Cube..................	20.4×10^{-4}	558×10^{-7}	36.6
Leslie Cube..................	25.2×10^{-4}	682×10^{-7}	36.9

The consistency of the values in the last column show that the results obtained by " thermo-level " technique are correct in absolute value as well as relatively.

The technique of making the skin temperature measurements is similar to that for making the calibration. The radiometer is pointed at the *R.T.B.* while the galvanometer index is set to zero and the temperature of the *R.T.B.* is recorded. The instrument is then placed over the part of the skin whose temperature is desired. The full aperture of the radiometer which is included within the four amber tips (the area included within a circle 5 cm. in diameter) must be filled in order that the measurement can be made. This area, of 20 cm², is arbitrary and by altering the design, can be made smaller (10 mm²), or larger as desired. The present arrangement is not adapted to the measurement of the skin temperature of a single finger or toe or other limited area except as averaged in with the surrounding areas. As soon as the instrument is placed on the skin the galvanometer will deflect and after twenty seconds will reach a point of equilibrium. The deflection is balanced to zero by means of the potentiometer whose reading, in calories, is recorded. The calorie readings can then be converted into temperature by means of the slide rule mentioned before.

The detailed measurements on normal subjects included in the stimulating papers of Bohnenkamp and Ernst (6) offer the only possibilities in the literature for a comparison of measurements made by the radiometer with those by other investigators. Bohnenkamp and Ernst point out the fact that the radiation from the forehead is very constant in all normal per-

THE RADIATION OF HEAT FROM THE HUMAN BODY

II. A Comparison of Some Methods of Measurement

By JAMES D. HARDY

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In this paper it is proposed to discuss some of the more usual methods for making measurements of skin temperature. Four instruments of different design have been tested under a variety of experimental conditions and the comparative results are offered with explanations of the differences which are found to exist between the several methods. The designs investigated are those of F. G. Benedict (1), G. F. Soderstrom (2), the Taylor Instrument Company (3), and the radiometer described in the preceding paper (4). These instruments are believed to be typical of the many devices which have been developed for this work, hence the conclusions drawn from the results of these tests are applicable generally to instruments of similar design.

Probably the most important work on the technique of measurement of surface temperature is that of Aldrich (5) who made comparative measurements of surface temperature with a surface thermocouple thermometer and with a radiation device. Recently Grassberger (6) has made some tests on the theory of thermocouple thermometers but his results do not include comparative data. The latest work on this subject is that by Bedford and Warner (7) who compared several surface thermometers with a radiometer by making measurements on the human forehead. Their conclusions are in general agreement with the present findings, although comparisons made on the skin surface have the obvious disadvantages mentioned below. Aldrich's experiments are so complete that they will be discussed here in detail in order to illustrate the difficulties encountered in the measurement of surface temperature. Aldrich made measurements on 20 persons using both the thermocouple and radiometer and his results showed that the readings of the thermocouple device were systematically *lower* than those of the radiometer by about 1.1° C. In a supplementary report (8) he endeavored to ascertain wherein this difference lay and his conclusion was that his thermocouple device was more to be trusted than his radiometric device. If one consider this disagreement from a physical standpoint there immediately arises an inconsistency. There can be no doubt but that Aldrich's radiometer (9) was actually measuring with sufficient

precision the energy radiated from the skin or clothing. The question arises as to the whether or not he was justified in transforming, through the Stefan-Boltzmann equation, his radiometric energy values into surface temperatures which can be compared with those obtained from his thermocouple. One may examine this question in the following way:

The radiation formula which expresses the exchange of radiation between two bodies at different temperature is

$$Q = k \, e \, e' \, S_0 (T^4 - T_0^4),$$

where, Q = the energy exchange,

k = proportionality factor depending upon the size, shape, and localities of the bodies,

e = absorbing power of the receiver,

e' = emitting power of the emitter,

S_0 = Stefan-Boltzmann constant,

T = Absolute temperature of the emitter,

T_0 = Absolute temperature of the absorber.

In Aldrich's radiation experiments all the constants of the equation were known except e' and T, and therefore if he were solving for T, the skin temperature, it would be necessary for him to assume a value for e', the emissivity of the skin. He based his calculations upon the assumption that the human skin is a perfect radiator, thus making $e' = 1$. If, however, he should substitute the values of T as determined by means of his thermocouple thermometer into the above equation and solve for e', it is obvious that e' would have a value greater than unity. This would indicate that the skin radiated more perfectly than an ideally perfect radiator. This, of course, as was recognized by Aldrich, is physically impossible. So far as I am aware, this is the only attempt to compare the radiometric method with the thermocouple method and it shows beyond question that Aldrich's thermocouple thermometer was not measuring the temperature of the surface from which the radiation was emanating. There seem to be only two interpretations of the matter, either the thermocouple thermometer was not reading the correct surface temperature, or the radiometer was reading the temperature of layers of tissue below the actual surface of the skin. This latter, indeed, is what Aldrich tentatively assumed to be the case, suggesting that the outer layers of the skin are transparent to infra-red light and that, therefore, the radiometer was " seeing " down into the deeper and warmer layers. He also suggested the possibility of " skin being so rough that the thermocouple could come in contact wit outer, cooler, ridges, while the radiometer a ged in the alleys." This latter suggestion seems har ice one mig hit a valley about as often as a ridge an ces theref ned ou e evidence as to the forme is briefly

According to Bazett and McGlone (10) the tissue 4 mm. below the surface of the skin is about 1.1° C. warmer than the surface, therefore should the radiometer see down to approximately this depth the discrepancy observed by Aldrich might be accounted for. Aldrich, however, in a later report (8) finds that no infra-red light is transmitted through a layer of skin 2 mm. thick. The question of the infra-red absorption of skin is now under investigation in this laboratory by Dr. C. Muschenheim and myself, and our preliminary results indicate that 95 per cent of the infra-red beyond 5 μ is absorbed by a layer of skin 0.2 mm. thick. Thus if the radiometer were reading the temperature of the layers of tissue 0.2 mm. below the surface, we might expect that it would read 0.06° C. higher than a thermocouple which is reading the actual temperature of the surface. Thus it would seem from this work and that of others that the heat is conducted to the outer layers of the skin and radiated therefrom in the form of heat waves.

Another important point in connection with the estimation of skin temperature from radiation data is the value of e', the emissivity of the surface of the skin. This matter is taken up in detail in Part III of this series (12), and it will be sufficient here to say that all the evidence which has been obtained by others and myself indicates that the skin radiates so well as to be a perfect radiator within the error of measurement in such experiments. Therefore it would seem that the calculation of skin temperature from the unmodified Stefan-Boltzmann formula, once the energy has been correctly measured, is a legitimate procedure. That being the case, the fault in Aldrich's experiments must have been with his thermocouple thermometer and not with his radiation device. Having had already some evidence to support this thesis I repeated the experiment which had led Aldrich to the contrary conclusion. Aldrich's experiment is briefly described.

A copper calorimeter supplied with a stirrer and thermometer had three holes cut in its side and over these holes were cemented rubber diaphragms of thicknesses varying from 0.1 mm. to 1.2 mm. With his surface thermo-element applied to the outside of the diaphragms, Aldrich made readings of the temperature of the surface of the rubber membranes. These data, together with the thicknesses of the diaphragms and the temperature of the calorimeter water and room temperature, enabled him to plot diaphragm thickness, d, as abscissae, and, ΔT, the differences between two sides of the diaphragm, as ordinates, for various differences in temperature between the calorimeter and the room. The results of his measurements are shown in Figure 1, Curves A and B. Curve A represents the relationship when the difference between the calorimeter temperature and room temperature, $\Delta T'$, was 10° C.; Curve B when $\Delta T' = 5°$ C.

The theory of heat conduction leads one to expect the curve between diaphragm thickness and ΔT to be of the general shape shown by A and B, that is, an exponentially increasing ΔT which gradually approaches $\Delta T'$ as

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The theory of heat conduction leads one to expect the curve between diaphragm thickness and ΔT to be of the general shape shown by A and B, that is, an exponentially increasing ΔT which gradually approaches $\Delta T'$ as

a limit for very large thicknesses of rubber. It is worthy of note, therefore, that for such small thicknesses of rubber, Curves A and B so rapidly approach their apparent limits and that these limits are so far removed from the actual values of $\Delta T'$. This evidence together with that before
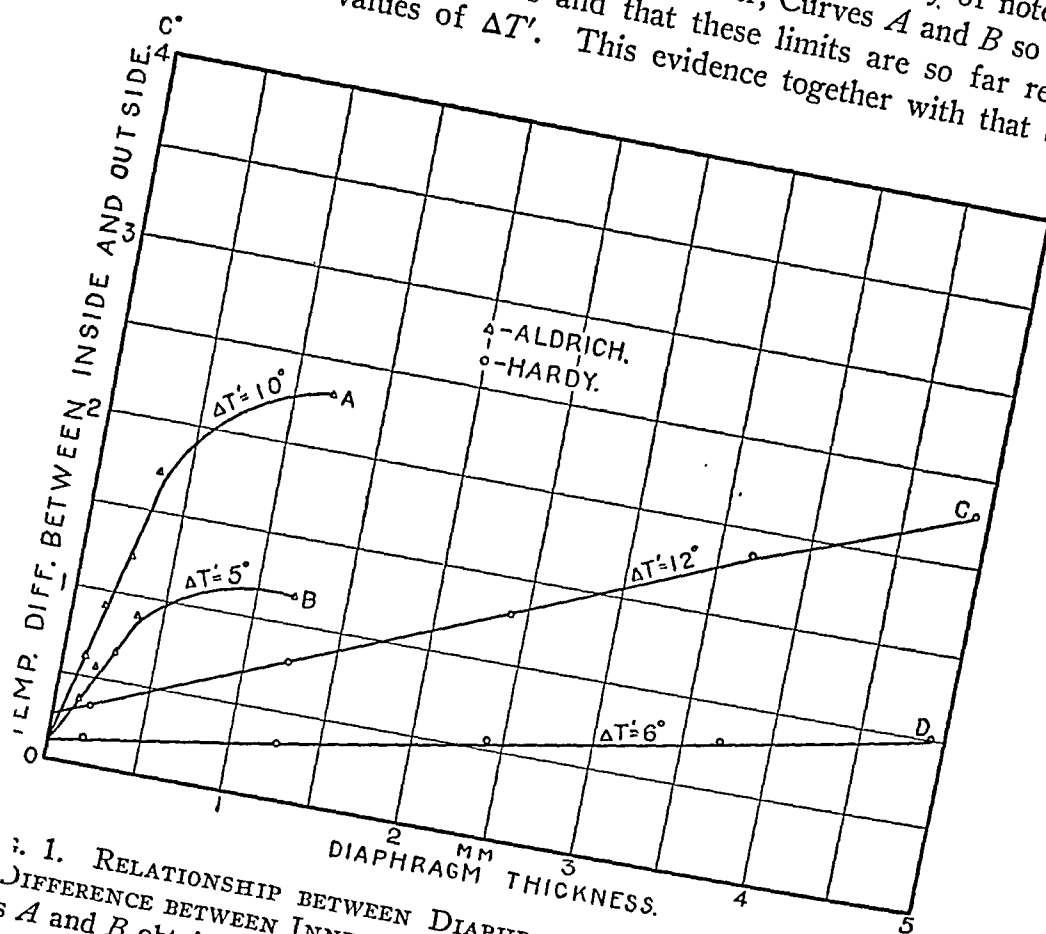
Fig. 1. Relationship between Diaphragm Thickness and Temperature Difference between Inner and Outer Surfaces of the Diaphragms. Curves A and B obtained by Aldrich; C and D obtained with radiometer.

mentioned made the repetition of the experiment seem worth while, and it was undertaken with the following differences: The diaphragm thicknesses were extended from 0.18 mm. to 5 mm. and the outside surface temperature was measured by means of the radiometer. The results are shown in the same figure, Curves C and D. The value of ΔT rises almost linearly with d until $d = 3.7$ mm. where indications of the exponential character of the curves begin to be manifest. An extrapolation of Curve C (with constant slope) would make $\Delta T = \Delta T'$ when $d = 25$ mm., whereas an extrapolation of Aldrich's Curve A would lead to the incongruous result of $\Delta T = 3°$ C. for very large thicknesses of rubber. Aldrich concluded from the fact that the extrapolated Curves A and B pass almost through the zero of ΔT for $d = 0$, that his thermoelements were reading the correct surface temperature, but while this is a necessary condition it is not a sufficient one and the slope of the curves may be changed at will by altering the external conditions.

It will be noticed that upon extrapolation to zero diaphragm thickness the Curves C and D do not pass through the point of zero ΔT, which means that the radiometer temperatures for the rubber are too low. This is due to the fact that rubber is not a perfect black-body. If, however, the curves be corrected for the emissive power of the rubber (0.98) they then pass through the zero point. The corrected curves give the true values of ΔT from which the surface temperature of the diaphragm can be determined. Upon comparing A and B with C and D, it will be seen that the thermo-element is indicating that rubber radiates more than a perfect black-body. An explanation of Aldrich's curves may be as follows: the too rapid rise in ΔT for thin diaphragms is probably due to the " calibration error " discussed below, an error which would tend to make the temperature readings too low; the too rapid sloping off of his curves is probably due to different conditions of thermal contact between the thicker rubber and his thermo-element. Experimental tests show that these two suggestions give approximately the right corrections to bring the thermocouple and radiometer into agreement.
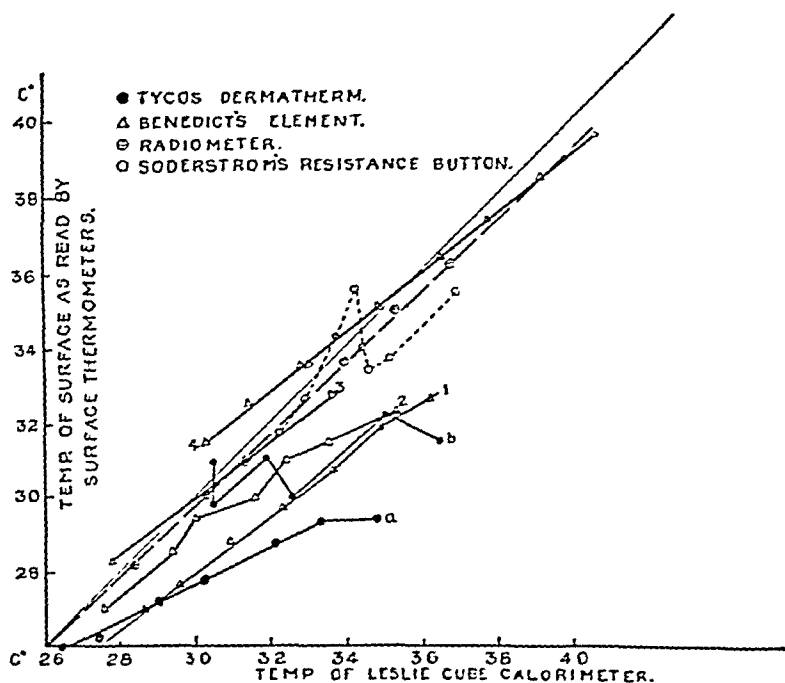
FIG. 2. TESTS OF SURFACE THERMOMETERS AGAINST A THIN RUBBER DIAPHRAGM

Having satisfactorily accounted for Aldrich's tests it became obvious that similar tests upon other designs of thermoelements were desirable in order to determine whether or not the matter of design was of major im-

portance.   Therefore the skin thermometers of Benedict, Soderstrom, and The Taylor Instrument Company were compared against the radiometer. These tests were made on the thinnest rubber diaphragm, the inside temperature of which was assumed to be that of the calorimeter water and the outside surface temperature was calculated from the experiment just described.   The results are shown in Figure 2.

The calibrations of all the devices were checked before the tests, and the method of applying the thermometers to the diaphragm was varied so as to include several possibilities.   The usual method, developed by Benedict, of first warming the thermometer with the thumb, then placing the element on one edge of the diaphragm and lastly slipping it over to the middle as soon as equilibrium was established. was considered standard.

The diagonal line represents the temperature of the surface of a hypothetical diaphragm of zero thickness; the broken curve gradually diverging from this line represents a corrected curve for diaphragm thickness 0.2 mm., and it is to this curve that all the other surface thermometers are referred.   Four sets of values are given for Benedict's thermocouple (designated by the triangles).   Number 2 represents a test at room temperature 23° C. and Number 4 at 33° C., using the standard method of application. (Curve Number 4 should be referred to the diagonal line rather than to the corrected curve which is valid only for temperatures near 23° C.)   The effect of room temperature upon the readings of the Benedict thermometer is obvious.   A great many tests were made with the Benedict element and it was found that the readings on the diaphragm could be changed almost at will by using different methods of applying the element.   Curve 1, with Benedict's device, was obtained by clamping the element in contact with the diaphragm and then raising the temperature in the calorimeter.   Curve 3 was obtained by keeping the device well heated in the closed palm of the hand between measurements but following the standard procedure as to the application of the device to the diaphragm.   The effect of the temperature of the backing of the sensitive element is thus brought out.   This emphasizes the point made by Benedict that a standard procedure of application be adopted, but it was found that even using such a procedure the readings were not consistent to better than ± 0.5° C.   The irregular curves shown for the Tycos " Dermatherm " and the Soderstrom resistance button were obtained by warming the calorimeter and, after making a reading, cooling it down rapidly.   The large heat capacities of the instruments are thus shown to cause a reading to be affected by the previous one, if the readings are made in the usual rapid manner.   If the observations are made slowly enough the irregular character is much reduced and the instruments show qualitatively the same characteristics as the Benedict element, the " Dermatherm " showing larger errors and the resistance button somewhat smaller.   Curve " a " for the " Dermatherm " was made by clamping the device to the diaphragm.   The radiometer was tested many

times under the same conditions as the surface thermometers and its readings showed good agreement ($\pm 0.1°$ C.) each time.

The errors generally encountered in the use of skin thermometers might be classified as follows, errors due to calibration, errors due to manipulation and errors due to the effect of the instrument on the surface to be measured. The first two sources of error, while troublesome and probably present in the work of past observers, can be partially circumvented under special circumstances; the last source is inherent in the methods and while generally much smaller than the first two would probably prevent the methods from ever becoming precise. The magnitude of these combined errors may total as much as four or five degrees centigrade depending upon conditions.

The "calibration errors" refer to the errors induced in the measurements made by surface thermometers due to the difference between the calibrating and measuring conditions. This error is believed by the present author to be the largest of the three mentioned above. The usual calibration procedure for surface thermometers is that of comparing the surface thermometer and a standard thermometer while submerged in some liquid. The thermal-junction is then surrounded by the liquid on all sides and is certainly at the temperature of the liquid (and of the standard thermometer) as the calibration is carried on. When the instrument is brought out for use in the measurement of skin temperatures the conditions are widely different from those of the calibration. In this latter case only a part of the sensitive element of the thermometer is in contact with the skin while the remainder is either in contact with the cool room air, as in Aldrich's device, or in contact with some other surface generally cooler than the skin, as in the Benedict and Tycos devices. It is evident that the thermocouples will assume a temperature somewhere between that of the skin and that of the external medium and therefore will not measure the true surface temperature. This probably accounts for the fact that the usual surface thermometer gives temperatures lower than that of the radiometer. This error should be systematic if sufficient precautions are taken to eliminate other sources of error and the surface thermometer should be expected to be in error by about $-1.0°$ C. when used at a room temperature of 20° C. to 25° C. The error may become positive when the thermometers are used at high room temperatures, and also at low room temperatures when certain methods of application are used.

Errors due to manipulation are those arising from the fact that the equilibrium point of the thermometer depends, among other things, upon room temperature, the excellence of the thermal contact (pressure with which the element is pressed against the surface, moisture of skin, etc.) design of instrument, method of applying the thermometer, etc. These errors are probably small when one person is making readings under a given set of conditions with a given instrument in a given manner, but they

become very much larger when measurements are to be made for comparison in other laboratories when using a different or even the same instrument. This fact makes the work of any single observer unique, not amenable to check or confirmation, and reduces the value of skin temperature measurements to simple experiments of differentiating a hot spot on the skin from a cold one qualitatively. It is recognized that such measurements are now made and are of value clinically. However, a systematic investigation of value to all observers is not possible using the usual skin thermometer, and it might be for this reason that the temperature of the skin is scientifically of no more value than it is at present.

The errors due to the effect of the instrument on the skin are hard to separate from the two former. They have been discussed by Cobet and Bramigk (11) and by Bazett and McGlone (10), but it is probable that this is the smallest source of error when using devices of small heat capacity.

It is not the purpose of this article to condemn the time-honored methods for making measurements of skin temperature, but rather to present them in their true light to indicate those fields for which they are best suited. The general ruggedness and simplicity of these instruments make them ideal for experiments that do not require a relative accuracy greater than $\pm 0.5°$ C. or an absolute accuracy greater than $\pm 1.0°$ C. Recognizing these general limitations the fields of investigation to which the several methods are applicable become clear, and investigators can choose an instrument which will best fit the case.

The experimental material included in this paper is not regarded as absolutely conclusive in regard to the accuracy of the skin thermometers for the very reason that the data are taken from a rubber diaphragm and not the skin. However, the evidence gained therefrom, taken together with the difficulties discovered by Aldrich, point to the fact that there is little to be expected from the difference between the skin and the rubber diaphragm. The skin does not make a good surface upon which to test instruments of this kind because its temperature is not known independently and its temperature is not constant either as to time or locality. The thin rubber membrane, on the other hand, simulates the skin surface and its temperature is constant over its surface for a sufficient length of time to make tests. Furthermore, the surface temperature is known (even without corrections for diaphragm thickness) to a sufficient accuracy and can be controlled for testing purposes. Many tests of the skin thermometers were made, however, on the skin and compared with measurements made with the radiometer. The results of these tests confirmed the observations of Aldrich when the tests were made at room temperatures near 23° C. It was found that by using various methods of applying the thermometers, thermocouple readings were obtained either higher or lower than the radiometer readings. This was particularly true of all instruments which had more or less massive

coverings to their sensitive elements, i.e. the Benedict, Soderstrom, and Tycos elements. The tests on the skin, however, can only indicate whether or not the skin thermometers are in agreement but do not give any clue as to which instruments are reading accurately the skin temperature. The evidence whether obtained from the skin or from the rubber diaphragm is in complete agreement as far as this most important question is concerned, and supports in every detail the conclusions enumerated above.

SUMMARY AND CONCLUSIONS

1. A repetition of the studies of Aldrich indicates that the discrepancies between his radiometer and skin thermometer readings were due to errors in the thermocouple thermometer, these errors being inherent in all skin thermometers which depend on contact with the skin surface.

2. The results of tests on the surface thermometers of F. G. Benedict, G. F. Soderstrom, and the Taylor Instrument Company show that the thermometers read too low by 1° C. to 3° C., depending upon conditions, when used at room temperatures in the neighborhood of 20° C. The tests show also that the reproducibility for the skin thermometers is in general about ± 0.5° C. and this error may run as high as ± 5° C. if readings are not made under uniform conditions with a standard technique.

3. The errors in measurements of skin temperature made by the usual type thermometer are classified as "calibration errors," "manipulation errors," and errors due to the effect of the instrument on the skin. It is concluded from a consideration of these errors that a relative accuracy of ± 0.5° C. and an absolute accuracy of ± 1.0° C. is the best that can be expected from instruments of similar design.

4. Skin temperature measurements with a radiometric device are shown to be accurate both relatively and in absolute value to ± 0.1° C.

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THE RADIATION OF HEAT FROM THE HUMAN BODY

III. The Human Skin as a Black-Body Radiator

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According to the law of Kirchhoff the ratio of the radiating power to the absorbing power is the same for all bodies at the same temperature, and therefore a body which absorbs perfectly must radiate perfectly. For that reason a perfect radiator is often referred to as a perfect black-body. All physical bodies radiate somewhat less than a perfect black-body. Surfaces such as polished metal radiate practically nothing whereas other surfaces such as black paint pigment radiate within a few per cent of the theoretical black-body. The human skin has its place among other radiators as regard its emissive power and upon the value of this quantity depends the whole technique of skin temperature measurements by means of radiometric instruments. This factor is of importance in the study of human radiation in general and, incidentally, in the study of ventilation. The human skin has been assumed in the past to radiate as a perfect black-body and the experiments of Cobet and Bramigk (1) confirm this assumption. Realizing the importance of this factor in the measurements of skin temperature when using a radiometer several experiments were performed with an idea of making a quantitative measurement of the emissive power of the skin. These experiments are described below and they do little more than confirm the observations of Cobet and Bramigk.

That the human skin is not truly black, whether white or negro, is obvious and the measurements of Martin (2) show quite conclusively that the reflecting power of even the blackest skin is considerably above zero. The question may then arise as to how the white skin can radiate so perfectly when it is obviously not black. A consideration of Figure 1 will make this clear. Here are plotted the emission curves of the sun (not to scale), a red-hot stove, and the human skin (to scale), assuming the emissivity of the skin to be 100 per cent. The electromagnetic spectrum is plotted somewhat schematically to include all the known radiations, and is to scale only for the visible and near infra-red regions. Due to atmospheric absorption none of the radiation from the sun of wave length longer than 3 mu reaches the earth's surface. On the other hand the human body has no appreciable radiation of wave lengths shorter than 4 mu. Thus the
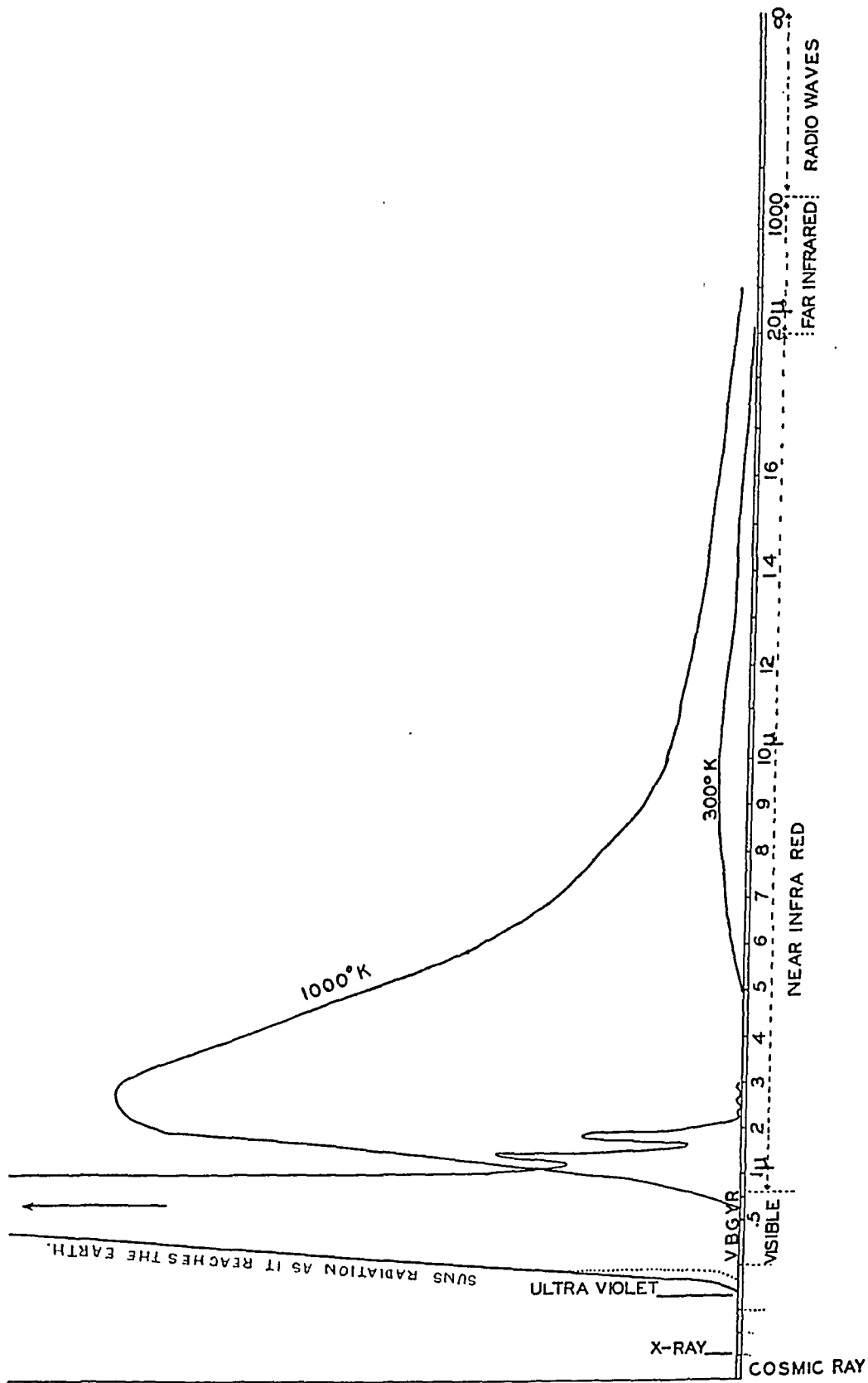
FIG. 1. SPECTRAL DISTRIBUTIONS OF THE RADIATION FROM THE SUN—RED HOT STOVE—AND THE HUMAN BODY

ability of the skin to reflect light of short wave lengths does not preclude the possibility of its absorbing and therefore radiating almost perfectly the longer wave lengths. Therefore the findings of Martin and of Cobet and Bramigk are not in contradiction to Kirchhoff's law. As shown above the regions of body emission and solar reflection do not even overlap. A consideration of the hypothetical emission curve for the skin shows that the visible color of the surface is of no importance in so far as the radiating power of the skin is concerned. To test this assumption a more or less qualitative experiment was performed as follows:

A white subject, parts of whose back had been coated with an absorbing black paint, was measured several times and comparisons were made between the radiation values of the blackened and unblackened surfaces. No significant difference could be detected between the two although the unblackened surfaces radiated slightly more than the blackened surfaces.

In Figure 2 is shown the emission curve for a perfect black body at a temperature of 32° C. or 305° K radiating to surroundings at 22° C. or 295° absolute, drawn on a larger scale than in Figure 1. Starting with this curve it is possible to make some investigations as to the closeness with which the emission curve of the skin follows the theoretical one for a blackbody. In order to test this several crystals whose transmission curves are well known were chosen and the total amount of energy transmitted through the crystal compared with the amount absorbed. The materials chosen were glass, crystal quartz, clear KCl, and NaCl. The transmission curves of these materials are shown in the same figure after having been corrected for the thicknesses of crystal used in this experiment. The glass was 1 mm. thick, the quartz 1 mm. thick, the NaCl 4 mm. thick and the KCl 2.5 mm. thick. Thus by multiplying the emission curve by the transmission curves and using graphic integration the amounts absorbed can be determined. This was done and the results compared with the experimental values obtained for the skin and an experimental black-body. The comparison is set forth in Table I.

TABLE I

Comparison of radiation from black-body and from skin

Substance	Per cent of incident light transmitted		
	Calculated	Experimental black-body (cone of Leslie Cube)	Skin
	per cent	*per cent*	*per cent*
Glass....................	0	0	0
Quartz...................	0	0	0
NaCl....................	78	78	78
KCl....................	87	88	87

This experiment is not particularly sensitive and is of more value in locating the position of the skin radiation in the spectrum than it is as a

quantitative test of skin blackness. From it the following conclusions may be drawn: (1) The emission curve of the skin must have approximately the same form as that of a black-body with no large regions of selective absorption or emission: (2) Radiation from the ordinarily moist skin is not greatly affected by the absorption by water vapor or CO_2 in the layer of air immediately surrounding the skin.
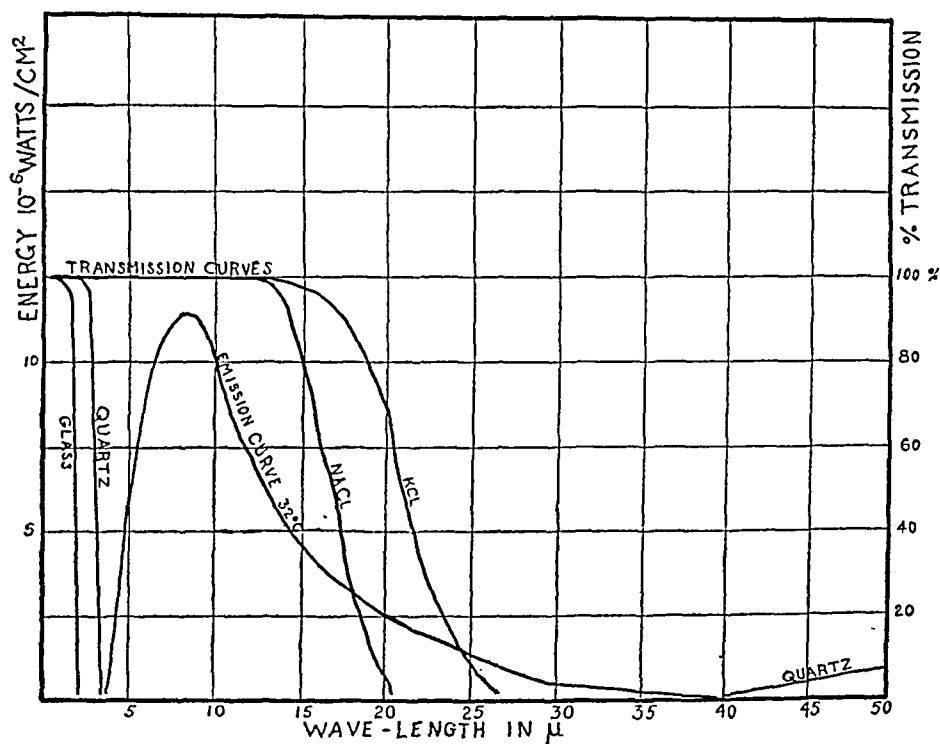
FIG. 2. CRYSTAL-FILTER TEST OF THE RADIATION FROM THE HUMAN BODY

Another, and much more sensitive method for measuring the radiating power of the skin, is that of comparing the radiation of the skin with that of a black-body emitter at the same temperature. In order to test the sensitivity of the method, experiments were performed on various surfaces of known temperature in the following manner.

The Leslie Cube, described in Paper I of this series (3), was arranged so that one surface of the cube contained the cone, or standard black-body emitter; into another side was drilled a hole over which was pasted a thin rubber diaphragm; a third side was painted black with a carbon black; and the fourth side was allowed to retain its usual dull copper finish. The water in the cube was warmed to about skin temperature, 35.8° C., and the radiation from all four surfaces was measured with a radiometer. Table II gives the comparative data for the experiment.

The sensitivity of the method is evident. The accuracy of the radiometer is ± 0.1 calorie. It is therefore sensitive to at least a 1 per cent change in the radiating character of the surface. The last column shows the black-body temperature of the surface whose actual temperature is

given in the first column, thus it is possible that a body might be so highly reflecting as to lose nothing by radiation regardless of its temperature. One of the important properties of clothing brought out by such an experiment is that of changing the radiating surface which man presents to his usually cooler surroundings.

TABLE II

Influence of character of surface on radiation

Surface	Cube temperature	R.T.B. temperature	Radiation		Black-body temperature
	° C.	*° C.*	*cal/sec/cm.²*	*per cent*	*° C.*
Cone..........	35.8	25.6	15.6	100	35.8
Rubber.........	35.8	25.6	15.3	98	35.6
Blackened.......	35.8	25.6	14.4	93	35.0
Copper.........	35.8	25.6	1.4	10	26.5

The next matter is that of actually measuring the temperature of the skin by a method independent of the radiometer so that the skin temperature can be compared with the black-body temperature, as measured by the radiometer. This was attempted in two ways.

The errors induced in the measurements of skin temperature when using the usual methods have been described (4), and the largest of these was the calibration error. In order to avoid this error the room temperature was raised until it approximated that of the skin. Under these conditions the skin temperature was measured with Benedict's element.

At this temperature the skin and all the apparatus were in approximate equilibrium and the maximum reading of the thermocouple thermometer was 0.7° C.

The Stefan-Boltzmann Law,

$$S = EE' \, S_0(T^4 - T_0{}^4),$$

relates the radiating power of the surface to its temperature. In this equation

S = number of calories radiated by the surface per second per $\overline{cm}^2$ is measured with the radiometer,

E = absorbing power of radiometer and does not enter the calculations (see Paper I (3)),

E' = the absorbing (emitting) power of the skin,

T = absolute temperature of the skin measured by the Benedict thermocouple,

T_0 = absolute temperature of the R.T.B.

Now, if T, as calculated on the basis of $E' = 1$ (known as the equivalent black-body temperature), should equal T as measured by the Benedict thermocouple. E' would necessarily be near unity. The accuracy of the experiment is limited by the precision with which the thermocouple thermometer will measure the skin temperature.

The readings were made on a white subject who did not sweat profusely at this temperature, and the usual complications met with in the use of the thermocouple thermometers seemed to be overcome under these special circumstances. The skin temperature was measured in several points and compared with that of the radiometer. The radiometer gave as an average of ten readings a skin black-body temperature of 36.1° C. and Benedict's element an actual 36.1° C. The room temperature was 35.8° C.

Another method for measuring the skin temperature was that which has been used by Cobet and Bramigk. A mercury thermometer is used to measure the temperature of the axilla. As soon as the thermometer has reached equilibrium the arm is moved and the temperature of the skin surface is measured as rapidly as possible with a radiometer. This experiment was repeated with the result that the mercury thermometer showed an average axilla temperature of 35.8° C. and the radiometer a skin temperature of 35.8° C. Thus this method, as sensitive as it is, does not give a value for the emissivity of the skin which is appreciably less than unity.

The whole of the evidence concerning this characteristic of the human skin points to the fact that the skin is as near a black-body as can be detected by experiment. This applies to the white skin as well as to the black and the validity of the radiometric method for skin temperature measurement is sustained on this score.

SUMMARY AND CONCLUSIONS

Additional evidence has been collected which supports the conclusion of Cobet and Bramigk concerning the emissivity of the skin for infra-red light. The value of the emissivity is put at 100 per cent with a possible error of 1 per cent.

Tests of the emission curve of the human skin show: (1) that it radiates like black-body irrespective of its visible color; (2) that the energy distribution in the spectrum is similar to that of an artificial black-body radiator; (3) that the presence of water vapor and CO_2 in the layers of air next to the skin do not appreciably affect the radiation from the surface.

The validity of skin temperature measurements by means of a radiometer is upheld as regards the emissivity of the skin.

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THE RELATION OF CIRCULATING ANTIPNEUMOCOCCAL IMMUNE SUBSTANCES TO THE COURSE OF LOBAR PNEUMONIA

I. NATURAL IMMUNE SUBSTANCES

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Interest in the humoral immune properties of the blood of patients with pneumococcus lobar pneumonia has centered principally on the possible relationship of acquired anti-pneumococcal activity of the serum to recovery from the disease. While much information has been collected on the subject by numerous workers, the nature of this relationship still remains problematical. Actually little has been added except corroboration to the results of Dochez's original investigation (1912) (1) in which he found that at about the time of crisis the blood serum of most patients recovering from pneumonia, in contrast to fatal cases, acquired the ability to protect mice against infection with highly virulent pneumococci. However, this immune property was not detected in every case and its appearance did not always coincide with the time of recovery. More recent studies have shown that the appearance of acquired antipneumococcal activity in the serum marks the termination of bacteremia [1] but whether this manifestation of the body's reaction is of primary or secondary significance in the mechanism of recovery, the present methods of investigation have failed to reveal.

A somewhat different conception of the problem was offered us by finding that the blood serum of normal human beings, as a group, possesses well marked pneumococcidal-promoting properties as shown by the in-vitro action of human serum-leukocyte mixtures (2).[2] The observation further that the serum of certain individuals lacked this property for one or more of the types of pneumococcus, while exhibiting it for the other types, suggested that there might be a relationship between the presence or absence of natural antipneumococcal humoral immunity and the inception of lobar pneumonia. A study of the serum activity of pneumonia patients, very early in the course of the disease, not only failed to disclose any such re-

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[1] The rarely observed exceptions to this finding will be discussed in Paper II.
[2] Similar observations were made at about the same time by Ward (3) and Sutliff and Rhoades (4).

TABLE II

Case 20 (F. V.) Hospital number 11423. Lobar consolidation of right lower lobe.
Pneumococcus Group IV isolated from lung and blood

Date..................April	24	25	26	27	28	29	30	May 1
Day of disease.................	4	5	6	7	8	9	10	11
Pneumococcidal - promoting action of patient's serum								
Fresh whole serum.......	0	0	10^{-7}	0	10^{-4}	10^{-4}	10^{-3}	10^{-3}
Heated diluted serum.....	—	—	—	—	0	0	0	0
Blood cultures.............	0	0	$+*$	0	0	0		
Evolution of lesion †								
X-ray..................	R2/3	=	=	=	=	>		
Physical signs...........			<Signs of beginning resolution					
Termination of disease......							<..Lysis...	.....>
White blood cells in thousands	4.0	6.0	7.5	8.8	19.0	24.5	14.0	13.0

† R2/3 signifies an x-ray shadow occupying 2/3 of the right lung field.
= indicates no change in the size or character of the x-ray shadow.
> indicates beginning clearing of the lesion.
* 8 colonies.

12 K. V.) showed a progressive spread into a new lobe at a time when resolution was occurring in the initially involved area. A fair degree of humoral immunity was present in the serum during this period.

BACTEREMIA

Our earlier studies on human cases of lobar pneumonia and experimentally infected animals suggested that there was a definite relationship between the presence of natural pneumococcidal-promoting properties in the blood and bacteremia. The present data make it necessary to qualify this inference to a considerable degree. In the present series of cases the occurrence of bacteremia was no more frequent in the patients lacking detectable humoral immunity than it was in those showing such serum activity. However, invasion of the blood stream was not found in those patients in whom the concentration of immune substances remained within normal limits. One exception to this finding was shown by Case 10 (W. S.) whose blood on one occasion yielded growth in the broth flask without growth on the plate and at the same time was found to possess a pneumococcidal-promoting power of 10^{-5} (10,000 pneumococci killed). Furthermore, a marked diminution or disappearance of humoral immunity during the course of the disease was usually followed by bacteremia. But exceptions to this finding were observed. Case 15, in whom the pneumococcidal-promoting properties of the blood had disappeared, showed a sterile blood 12 hours before death. Invasion of the blood stream was also noted
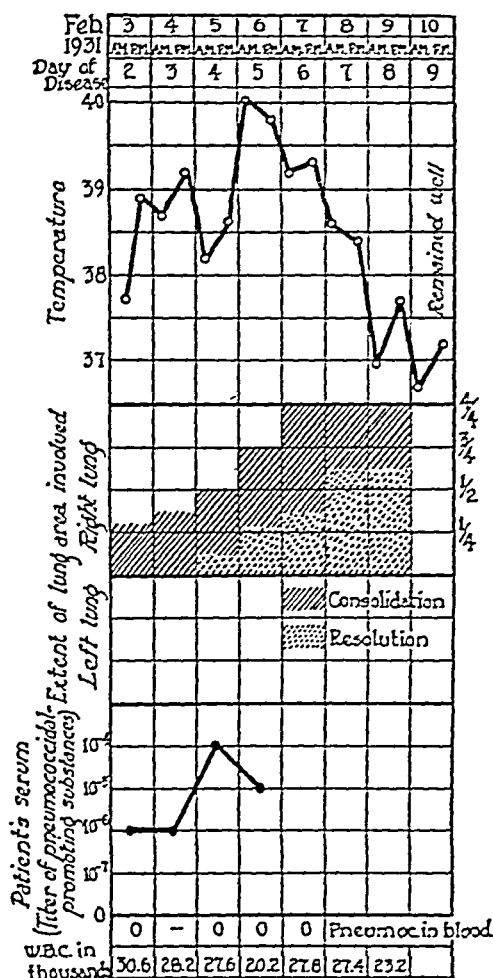
CHART II. CASE NUMBER 12 (K. V.). LOBAR PNEUMONIA, PNEUMOCOCCUS GROUP IV ISOLATED FROM THE SPUTUM

in two cases exhibiting a minimal concentration of immune substances. (Cases 1 and 9.)

Outcome of disease

Since the occurrence of pneumococcidal-promoting activity in the serum was found to bear no relationship to the inception of the disease (5), the spread of the pulmonary lesion or the invasion of the blood stream, one would hardly expect that any prognostic value as to outcome could be attached to presence or absence of this property in the blood. Our data show such to be the case. Patients exhibiting an initial absence of humoral immunity were just as likely to recover as those possessing it to a normal

degree. However, in all the fatal cases, with one exception (Case 9), the serum was found to be without immune activity at the end of the disease, whether or not it had been present earlier.

There were no significant differences in the number of circulating leukocytes between the patients with and without demonstrable humoral immune properties.

It will be noted in Table I that in a number of cases showing no serum pneumococcidal-promoting activity in the initial tests, this property appeared toward the end of the disease. Does this represent a reappearance of the natural immune substances or does it indicate newly acquired immunity? Since the data bearing on this question are presented in the next section, a discussion of the subject will be deferred until that time. Suffice it to say here that the evidence available points to the latter explanation.

DISCUSSION

In view of the observations just described, what conclusions are to be drawn concerning the relationship of natural immune substances to the course of lobar pneumonia? The one apparent effect of the persistence of a normal degree of pneumococcidal-promoting power of the serum, namely the maintenance of a sterile blood, was not without exception. The observations of Sutliff and Rhoades (11) indicate that bacteremia in the presence of well marked pneumococcidal activity of the blood is not of uncommon occurrence. While these authors made no attempt to differentiate between natural and acquired immune substances, their finding of pneumococcus-killing power in the blood, early in the course of lobar pneumonia, makes it highly probable that in such instances they were dealing with natural serum immunity.

In a discussion of the differences observed among the various animal species, including man, in respect to their ability to localize pneumococcus infection, Trask, O'Donovan, Moore and Beebe (12) present a diagram showing a curve dropping at the top from man through the child, immunized monkey, normal monkey, immunized rabbit down to normal rabbit at the bottom on an ordinate of the diminishing typical clinical picture of lobar pneumonia. The base line represents increasing pneumococcemia, most marked in the rabbit. Would it be possible to express pneumococcus susceptibility of other animal species in such manner? While adequate data is lacking in most instances, our recent production of experimental lobar pneumonia in the dog provides another instance of an animal with a resistance against the pneumococcus analogous to that of adult man and showing the ability to localize the infection in the lungs with the production of a lesion similar, in general, to clinical lobar pneumonia (13). We also found that the natural antipneumococcal immune substances, present in the dogs blood, often persisted in considerable concentration throughout the course of the experimental disease. Their disappearance was usually fol-

lowed by bacteremia and only rarely did blood invasion occur in the presence of humoral immunity. Just as was observed in human beings, extension of the lesion in the experimental pneumonia occurred whether or not immune substances were detectable in the blood.

Our present knowledge does not permit us to infer more than that those animal species possessing circulating immune bodies are endowed with the capacity to localize pneumococcus infection. Individuals of the species may lack such demonstrable immunity, but nevertheless be competent to localize the infective process. Tests on the common laboratory animals exhibiting a high degree of resistance to the pneumococcus including horse, sheep, dog, cat and pig, have shown them to possess natural antipneumococcal immune substances (10).[4] Whether these animals are all capable of localizing pneumococci in the lungs to produce the clinical picture of lobar pneumonia we do not know. However, lobar pneumonia does occur spontaneously in horses and we have produced in the cat (in a few instances) lobar pneumonia which appears to resemble the experimental disease in the dog. In the absence of complete experimental data, we are furthermore unable to draw any general conclusions concerning those animals which do not possess natural demonstrable circulating immune substances. The animals recognized as belonging to this group, the guinea pig and the rabbit, show very little ability to localize pneumococcus infection, but whether all animal species capable of localizing this microorganism show humoral immunity is not known. While tests for pneumococcidal activity in the blood of normal monkeys have not been reported, certain studies on the mouse protective properties of their serum suggest that demonstrable natural humoral immunity is either very slight or absent though lobar pneumonia can be produced readily in this species.

Thus quite apart from such possible species variations as may be presented by the monkey, the finding of individuals within a pneumococcus resistant group (exemplified by man) who do not possess demonstrable antipneumococcal immune substances, leaves no other alternative than to assume the existence of other unknown factors in the mechanism of natural resistance to the pneumococcus.

SUMMARY

A study of twenty-nine cases of pneumococcus lobar pneumonia was made with a view to determining the possible relationship of circulating natural antipneumococcal immune substances to the spread of the pneu-

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[4] In this study (10) human beings were classified erroneously as a susceptible animal. The cause of this error became apparent in later investigations in which it was found that there exists in normal human beings great variations in the concentration of natural immune substances in the blood. The several individuals tested in the earlier study showed an absence of antipneumococcal activity of the serum for the Type I Pneumococcus used.

monic lesion, the localization of the infection in the lung and the outcome of the disease. The immune properties of the blood were determined by testing the pneumococcidal-promoting action of the serum. Data on changes in the extent and character of the pulmonary process were secured principally from daily x-rays of the chest.

No relationship was found between the presence or absence of detectable humoral immunity and the extension of the lesion. Active spread of the process from lobe to lobe occurred in certain patients showing the persistence of a normal pneumococcidal-promoting power in the blood. In other cases in which the serum lacked this property the lesion showed no increase in size throughout the period of observation. Likewise the initial concentration of immune substances in the blood had no observable significance in relation to the outcome of the disease. Patients without detectable humoral immunity were just as likely to recover as those showing this property to a normal degree. In respect to the frequency of bacteremia, however, there was a difference between these two types of patients. With only one exception, the blood of those cases showing the persistence of a normal degree of pneumococcidal-promoting power, remained sterile, while bacteremia was of relatively frequent occurrence in patients lacking this property, initially or losing it during the course of the disease. On the other hand a number of patients without demonstrable serum immunity showed a persistent absence of blood invasion. We are thus left with the conclusion that the natural circulating antipneumococcal immune substances play at most a very minor rôle in the course of lobar pneumonia.

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THE RELATION OF CIRCULATING ANTIPNEUMOCOCCAL IMMUNE SUBSTANCES TO THE COURSE OF LOBAR PNEUMONIA

II. ACQUIRED IMMUNE SUBSTANCES

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In an earlier study of those factors concerned in the mechanism of recovery from pneumococcus lobar pneumonia, we found that the patients' serum at the time of crisis or lysis acquired the property of promoting the destruction of the homologous pneumococcus when tested in vitro with rabbit serum-leukocyte mixtures (1). Our results agreed essentially with the findings of others on the occurrence of mouse-protective action in the serum of patients recovering from this disease and presumably have the same significance. However, in our series of cases, the pneumococcidal-promoting activity of the serum occurred more regularly and was more closely associated with the time of beginning recovery than was the reported occurrence of mouse protective properties. Further observations on our part have failed to reveal such a uniform chronological connection with the termination of the disease. Not infrequently, heat stable pneumococcidal-promoting activity of the serum was detected 2 to 3 days before crisis or in certain cases, not until after this event. Thus, while a direct causal association between detectable humoral immunity and recovery cannot be inferred, it seems likely, in view of the nature of the action of the circulatory antipneumococcal substances, that certain changes in the course of the disease indicating increased control on the part of the body over the infectious process may be ascribable to this effect. The present investigation, which comprises a considerably larger series of cases than did the earlier one, was undertaken with a view to inquiring more particularly into the relationship which the occurrence of these newly acquired immune bodies might bear to the spread of the pulmonary lesion and the localization of the infecting organism in the lung.

METHODS

The technique employed for the detection of the acquired pneumo-coccidal-promoting properties of the serum was the same as that described

in former publications (1) (2). Briefly, dilutions of the test serum were added to constant amounts of rabbits serum and leukocytes which were then seeded with 10^{-6} of the standard pneumococcus suspension. This quantum contained 500 to 1,000 pairs of pneumococci and was uniform in each test. Serum samples from each patient heated to 56° C. for ½ hour were tested at one time with the homologous pneumococcus, or if the disease was caused by Type I, a highly virulent Type I strain, A_5, was employed. The virulence of the pneumococcus isolated from each patient was tested in adult rabbits and mice and if found to be of low virulence for the former, the serum and leukocytes from rabbits weighing about 500 grams were used, since the blood of rabbits at this stage of growth possesses no demonstrable pneumococcidal activity even for pneumococci of very low virulence for full grown animals (3).

In addition, part of each serum sample from a number of cases was kept unheated and tested for natural immune properties as described in the preceding paper (4). Mouse protection tests were carried out in a few instances both with fresh and heated serum. The opsonic and agglutination activity of the serum was also determined in several cases.

Blood cultures and serial x-rays were made on each case as described in Paper I (4).

CLINICAL CASES

Observations relating the appearance of acquired immune substances to the spread of the pulmonary lesion, blood invasion and outcome were carried out in thirty cases of pneumococcus lobar pneumonia. Twenty-four of these patients recovered.

The distribution of pneumococcus types among them was as follows: Type I, 1 case; Type II, 2 cases; Type IIa, 4 cases; Type III, 7 cases and Group IV, 13 cases.[1]

A summary of the findings in this group is presented in Table I.

Occurrence of immune substances

An examination of the data shown in Table I reveals the fact that pneumococcidal-promoting properties appeared or increased in the blood serum of all the patients recovering from the disease and in only one of the six fatal cases. In three cases, Numbers 1, 11, and 20, the heated diluted serum showed no pneumococcidal-promoting activity at any time while the fresh whole serum was found to possess this property to a well marked degree. All three serums showed mouse-protective action (see Table III). In two of these the immune properties were detected for the first time toward the end of the disease. In the other, demonstrable immunity had

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[1] The relatively small number of Types I and II in this series is due to the fact that patients with these two types were usually treated with serum and hence fall into the third part of the study.

TABLE I

Relation of acquired immune substances to course of disease

Case number	Type	Day of appearance of pneumococcidal activity		Day of beginning recovery or death	Spread of lesion after appearance of serum immunity	Blood invasion in relation to appearance of serum immunity	
		Heated diluted serum	Fresh whole serum			Before	After
1. (H. C.)....	III	None	4th*	R 3d	0	+	0
2. (E. C.)....	IV	1st (1 : 20)	1st	R 2d	+Δ	—	—
3. (G. B.)....	IV	5th (1 : 80)	—	R 4th	0	0	0
4. (R. M. E.).	IIa	5th (1 : 20)	3d**	R 6th	+Δ	0	0
5. (K. H.)....	III	4th (1 : 40)	4th	R 6th	+Δ	0	0
6. (L. G.)....	III	5th (1 : 80)	—	R 5th	0	0	0
7. (R. E.)....	II	1st (1 : 320)	1st	R 1st	—	0	—
8. (R. K.)....	IV	15th (1 : 40)	—	R 18th	+	0	0
9. (M. M.)....	II	7th (1 : 20)	7th	R 9th	0	0	0
10. (W. S.)....	IV	6th (1 : 640)	5th*	R 8th	0	+	0
11. (N. S.).....	II	None	10th*	R 9th	0	0	0
12. (A. R.)....	IV	10th (1 : 80)⊖	—	R 5th	0	+	0
13. (A. Z.).....	III	6th (1 : 160)	6th*	R 6th	0	0	0
14. (L. P.).....	IIa	3d (1 : 40)	3d	R 4th	0	0	0
15. (M. K.)....	IIa	6th (1 : 320)	—	R 6th	—	0	0
16. (R. B.).....	IV	7th (1 : 80)	6th	R 6th	0	0	0
17. (B. G.).....	IIa	5th (1 : 40)	5th	R 5th	0	0	0
18. (Y. B.).....	IIa	8th (1 : 40)	7th	R 6th	—	+	0
19. (M. K.).....	IV	8th (1 : 640)	5th	R 5th	0	0	0
20. (F. V.).....	IV	None	8th	R 9th	0	+	0
21. (A. L.).....	I	5th (1 : 40)	3d	R 6th	+Δ	0	0
22. (F. T.)....	IV	9th (1 : 20)	—	R 8th	0	—	—
23. (L. M. B.).	IIa	9th (1 : 80)	8th	R 8th	0	+	0
24. (R. R.)....	IV	6th (1 : 320)	—	R 6th§	. 0	0	0
25. (B. B.)....	III	9th (1 : 40)	—	D 11th	+Δ	0	0
26. (H. B.)....	IV	None	—	D 12th	—	+	
27. (B. D.)....	IV	None	—	D 8th	—	+	
28. (C. H.)....	III	None	None	D 10th		+	
29. (E. K.)....	IV	None	—	D 10th		+	
30. (S. R.).....	III	None	—	D 7th		+	

+ = present.

0 = absent.

— = no observations made.

R = recovered.

D = died.

The figures in column 3 (1:20), (1:40), etc. represent the highest dilution of the patients serum which, added to rabbits serum and leukocyte mixtures, caused death of all the pneumococci contained in 10⁻⁶ of the standard pneumococcus suspension i.e. 500 to 1,000 micro-organisms.

* These patients showed an increase in the pneumococcidal-promoting activity of the whole serum which had been present to a varying degree from the beginning of the observations.

** Patient was given Type II concentrated antibody solution.

§ Died 5 days later of nephritic uremia. Lungs at autopsy showed well advanced resolution of the lobes previously consolidated.

Δ = No new lobe involvement.

⊖ = Test on serum on 7th day negative. No further test until the 10th day.

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been constantly present, but showed a definite terminal increase. Whether the antipneumococcal activity found in the blood of these three patients represents the acquisition of new immune properties or only the recurrence of natural immune bodies which had diminished or disappeared during the active stage of the disease will be discussed later. It makes no essential difference in the chief inferences to be drawn from this study whether they are classified in one group or the other.

Relation of appearance of serum immunity to time of recovery

A tabulation of the recovering patients with respect to the day on which immune substances first appeared in the blood serum (Table II) shows no close relationship of this phenomenon to the onset of recovery. Less

TABLE II

Relation of appearance of serum immunity to recovery *

Kind of pneumococcidal test	Appearance of pneumococcidal-promoting action in relation to beginning of recovery						
	Before			Day of	After		
	3 days	2 days	1 day		1 day	2 days	3 days
Fresh whole serum Number of cases..............	3	2	2	4	2		
Heated diluted serum Number of cases..............	1	3	4	6	4	1	1

* This data is taken from Table I. Tests with the fresh whole serum were not performed on all the cases tested for acquired immune serum substances.

than half the cases showed the first appearance of serum immunity on the day of beginning recovery. The immune substances occurred more often before recovery than subsequent to it and in several instances as early as three days beforehand.

It is probably significant that in the two patients which showed an abortive type of pneumonia (Cases 2 and 7, Table I) acquired immune properties of the serum were demonstrable on the first day of the disease. Patient R. E., Number 7, exhibited all the classical symptoms of lobar pneumonia which lasted less than 18 hours. No evidence of consolidation was detected either by x-ray or physical signs, but a virulent Type II pneumococcus was isolated from the sputum. The pneumococcidal-promoting activity of the serum secured within a few hours after the onset of the disease was greater than that shown by the majority of patients at the time of recovery. However, the early presence of acquired immune substance is not always followed by prompt recovery as was found in Case 20, F. P. R. (Paper III, Table I) whose serum showed on the second day of the dis-

ease a pneumococcidal promoting titer of the same degree as that of Case 2 (E. C.) above mentioned, but which had disappeared by the fourth day. Recovery did not occur until the seventh day despite the restoration of immune properties in the blood by the injection of antipneumococcus serum.

Spread of the pulmonary lesion

While, in the majority of instances, there was no increase in the extent of the pulmonary lesion after the appearance of acquired immune bodies, a further spread of the process did occur in six cases despite the presence of demonstrable humoral immunity. In several patients the pulmonary lesion continued to enlarge for two to three days following the initial finding of humoral immunity, but in only one case, Number 8, was there detectable spread to a new lobe.

On the other hand the lesion remained stationary in its extent in several patients whose blood lacked demonstrable immune properties. (See Cases 19 and 20, Tables I and II, Paper I.)

Case 25, B. B., Chart I, the only fatal one showing the presence of acquired immune substances, deserves special comment. In this patient a progressive spread of the pneumonic process occurred throughout the period of observation from the fourth day of the disease until death on the eleventh day. On the ninth day the serum showed pneumococcidal-promoting titer of 1:40. This increased to 1:80 and persisted until the time of death. By the ninth day almost one half of the lung area was involved and by the eleventh day this had increased to three quarters. It seems likely in this instance that the accompanying marked anoxemia was an important factor in the exitus, since at autopsy much of the involved lung tissue was found to be in a state of resolution. No complications were discovered.

Bacteremia

In six of the patients (Table I) recovering from the disease, pneumococci were isolated from the blood at some time during its course. In all of these cases the blood cultures became negative either before or coincident with the acquisition of humoral immune substances. On the other hand there were two instances, Cases 18 (Chart II) and 20 (Table II, Paper I) in which bacteremia disappeared in the absence of detectable humoral immunity. It may be significant that the only patient going on to a fatal termination without demonstrable bacteremia, showed the presence of immune properties in his blood for several days before death.

Comparison of pneumococcidal-promoting activity of fresh whole serum and heated diluted serum
(Natural and acquired immune substances)

The particular composition of the pneumococcidal test employed in this section of the study was designed to distinguish acquired from natural im-

mune bodies. It was early discovered that dilution of fresh serum to $1:10$ was often not sufficient to abolish its pneumococcidal-promoting action and in order to bring about complete destruction of the pneumococci, the presence of approximately this concentration of fresh serum was essential.
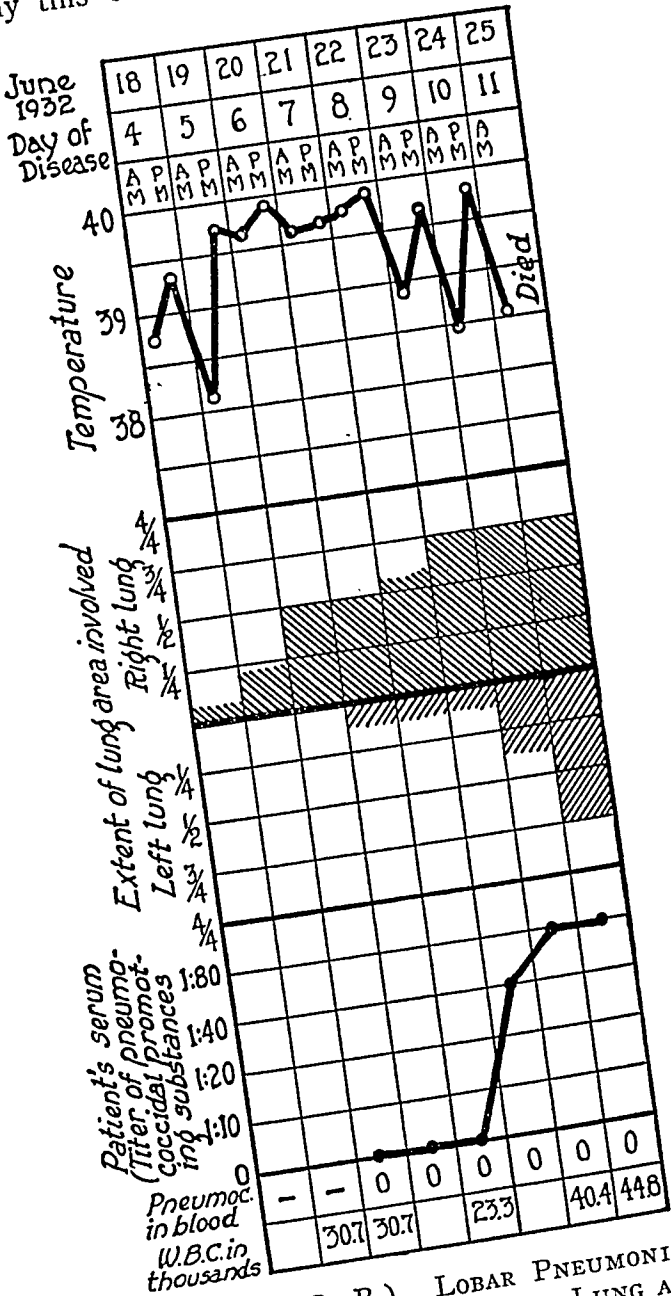
CHART I. CASE NUMBER 25 (B. B.). LOBAR PNEUMONIA, PNEUMOCOCCUS TYPE III ISOLATED FROM SPUTUM AND THE LUNG AT AUTOPSY

Thus, a human serum-leukocyte system could not be used for the detection of acquired immune substances unless these were present in high concentration. The difficulty was solved by the substitution of rabbits' serum and leukocytes and by inactivation of the human serum. When diluted

1 : 10 the inactivated normal human serum was without pneumococcidal-promoting action and at the same time produced no deleterious effect on the leukocytes. Concentrations of serum greater than 1 : 10 at times appeared to interfere with the normal functioning of the rabbit's leukocytes, hence this amount of serum was the maximum used in the tests, and to this extent the sensitivity of the test for low concentrations of immune substances is limited. In order to compare these two reactions a series of cases, in
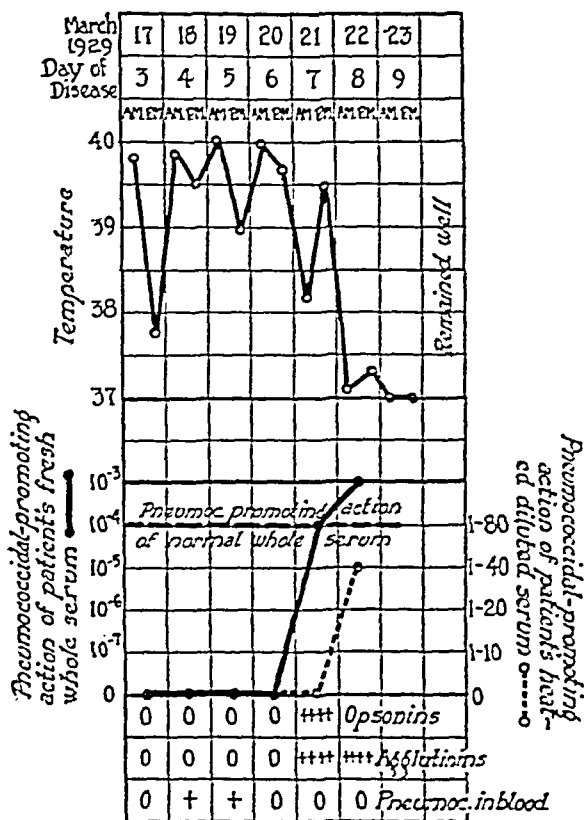
CHART II. CASE NUMBER 18 (Y. B.). LOBAR PNEUMONIA, PNEUMOCOCCUS TYPE II ATYPICAL ISOLATED FROM BLOOD

which both tests were performed on the same sample of serum, are listed in Table III together with paralleled tests for mouse protection. It will be noted, first of all, that there is little parallelism quantitatively between the results of the two pneumococcidal tests. Some differences might be expected, since in the experiments with whole serum the numbers of pneumococci are varied and the amount of human serum held constant, while in the tests with heated serum the number of microorganisms was kept uniform

TABLE III

Comparison of pneumococcidal tests with each other and with mouse protection *

Case number	Day of disease	Pneumococcidal tests		Mouse protection	Day of beginning recovery
		Heated diluted serum	Fresh whole serum		
1. (H. C.)..............	2d	0	10^{-7}	0	
	3d	0	10^{-6}	10^{-4}	3d
	4th	0	10^{-5}	10^{-5} (Inact. ser.)	
11. (N. S.).............	2d	0	10^{-4}	10^{-6}	
	4th	0	10^{-3}	10^{-7}	
	6th	0	10^{-4}	10^{-6}	/
	8th	0	10^{-6}	10^{-6}	
	10th	0	10^{-4}	10^{-7} (Fresh ser.)	9th
20. (F. V.).............	4th		0		
	6th		10^{-7}	0	
	7th		0	10^{-7}	
	8th	0	10^{-4}	10^{-5}	
	9th	0	10^{-3}	10^{-6}	9th
	10th	0	10^{-3}	10^{-6}	
	11th	0	10^{-3}	10^{-5} (Inact. ser.)	-
13. (A. Z.).............	3d	0	10^{-6}	0	
	4th	0	10^{-7}	0	
	5th	0	10^{-6}	0	
	6th	1 : 160	10^{-5}	10^{-7} (Fresh ser.)	6th -
16. (R. B.).............	5th	0	0	—	
	6th	0	10^{-5}	—	6th
	7th	1 : 80	10^{-4}	—	
10. (W. S.).............	4th	0	10^{-5}	—	
	5th	0	10^{-4}	—	
	6th	1 : 640	10^{-4}	—	
	7th	1 : 1280	10^{-4}	—	
	8th	1 : 640	10^{-4}	—	8th

0 = no activity.

— = not done.

* The figures 10^{-3}, 10^{-4}, etc. represent amounts of the standard pneumococcus suspension (1 billion micro-organisms per cc.) killed by the serum and leukocyte mixtures and under the heading " Mouse protection " they indicate the largest amount of injected pneumococcus suspension against which 0.2 cc. of the serum protected. 10^{-7} = approximately 100 pneumococci, 10^{-6}, 1000, etc.

TABLE III (*continued*)

Case number	Day of disease	Pneumococcidal tests		Mouse protection	Day of beginning recovery
		Heated diluted serum	Fresh whole serum		
21. (A. L.)...............	1st	0	0	—	
	2d	0	0	—	
	3d	0	10^{-5}	—	
	4th	0	10^{-4}	—	
	5th	1 : 40	10^{-5}	—	
	6th	1 : 640	10^{-3}	—	6th
15. (B. G.)...............	3d	0	0	—	
	4th	0	0	—	
	5th	1 : 40	10^{-6}	—	5th
	6th	1 : 320	10^{-4}	—	
14. (F.) (see Paper I).......	3d	—	10^{-5}	10^{-5}	
	5th	—	10^{-5}	10^{-7}	
	7th	—	10^{-4}	10^{-7} (Inact. ser.)	7th
27. (H. F. V. L.) (see Paper I)	5th	—	0	0	
	6th	—	10^{-5}	0	
	7th	—	10^{-5}	10^{-7} (Fresh ser.)	7th

with varying dilutions of the serum. However, the variation in results observed in Case 10, for example, in which the effective concentration of heated serum changed from 0 to 1 : 640 within twenty-four hours, while the numbers of pneumococci killed by the fresh whole serum remained the same on these two days, cannot be explained on the basis of our knowledge concerning the mechanism by which the pneumococci are killed by the serum and leukocytes.[2] The question of chief importance for the present consideration is that of the significance of the appearance of pneumococcidal-promoting action of the whole serum in patients whose blood, in the earlier part of the disease, has lacked this property. It was hoped that mouse protection tests would be of assistance, and the results of such tests do provide data of value since it will be observed that the pneumococcidal and mouse-protection activity of whole serum are of about equal sensitivity, the former being perhaps a little the more delicate of the two even when fresh serum is employed in both tests as in Cases 13 and 27.[3] In Case 20, both pneumo-

[2] It is possible that this phenomenon may be of the same nature as that observed in mouse protection experiments with immune serum—namely that when the infecting dose of microorganisms reaches a certain maximum, no amount of immune serum, however large, will protect the animal.

[3] Sutliff and Rhoades (5) detected not infrequently whole blood pneumococcidal activity in the absence of mouse protective action of the serum.

coccidal tests and mouse protection tests with fresh whole serum showed the appearance of serum immunity at about the same time while the heated diluted serum was without affect and remained so. An analogous result was evident in Case 1 in which there occurred a well marked increase of the pneumococcidal-promoting activity of the whole serum accompanied by the appearance of mouse-protection activity, while no action of the heated diluted serum was demonstrable.

Tests for opsonic action of the serum were informative. Case 18 (see Chart II) showed no serum opsonic activity until the seventh day of the disease which was the first day of appearance of pneumococcidal action of whole serum. The degree of opsonic action was much greater than that observed with normal serum. Activity of the heated diluted serum was not evident until the eighth day. The serum of several other cases showed increases in opsonic and agglutinative activity at about the time of recovery, but these reactions did not parallel strictly the changing intensity of the pneumococcidal and mouse protective properties of the serum.

In conclusion it may be said that in our experience the demonstration of pneumococcidal action in the mixture of heated human serum with rabbit serum and leukocytes indicates the presence of so-called acquired humoral immunity. The appearance late in the disease of pneumococcus-killing power in the mixtures of fresh whole human serum and leukocytes is probably indicative of the same reaction since such serum has been found to contain mouse-protection activity which was not present previously. However, in an instance such as presented by Case 11 (N. S.) where the blood showed pneumococcus-killing properties throughout the course of the disease and the mouse-protection action of the serum was minimal and not demonstrable with inactivated serum, one cannot draw any inferences concerning the nature of the increase in humoral immunity at the end of the disease. Finally, we wish to point out that this attempt to distinguish between natural and immune substances does not imply a conception of two separate immunological entities. There is considerable experimental evidence to indicate that they are one and the same, the difference being quantitative and not qualitative.[4]

DISCUSSION

That the appearance of circulating type specific antibodies may be regarded as an indication of the body's successful reaction against pneumococcus infection seems clear from both the clinical and experimental evidence at hand. Accumulating data also show that this manifestation of immunity to pneumococci is found with great regularity in patients recovering from lobar pneumonia (7) (8) (9). But it may not become ap-

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[4] The whole matter of the relationship between natural and acquired opsonins has been ably reviewed by Zinsser (6).

parent until days or even weeks after recovery. In a recent communication Lord and Persons (8) report two instances of repeated tests on the serum of two patients with pneumonia who failed to show mouse-protective action until the 20th and 25th days respectively, after the termination of disease. Moreover, the development of humoral immunity does not always insure recovery. There are records of a few cases which have progressed to a fatal termination despite the presence of immune substances in their serum. The course of the disease in most of these instances has been prolonged far beyond its usual limits. Lord and Persons report two such cases. One was found at autopsy to have a pneumococcus endocarditis, the other dying at the end of 23 days had an infected chest fluid. Trask and his associates (9) observed one case which showed slight mouse-protective action in the serum on the 11th day and died on the 17th day with a staphylococcus abscess in the lung. Clough reports one case dying with complications (10). Our case described above died on the 11th day with extensive consolidation of the lungs, but without complications.⁵

Perhaps of more significance than the presence of humoral immune substances is their absence from the serum in many instances until after the time of recovery, because it suggests that the body possesses other and highly effective means for restraining the spread of the pneumonic process, localizing the invading microorganism within the lesion and eventually terminating the infection. Our findings make it seem doubtful whether the presence of pneumococcidal-promoting or mouse-protective substances have anything to do with the control of the spread of the lesion within the lung, since extension of the pulmonary process was observed to occur in a number of instances after the appearance of humoral immunity (see Table I) and, on the contrary, the lesion remained static for days in patients whose serum was without detectable immune properties either natural or acquired. With respect to bacteremia, however, there is some positive evidence that acquired immune substances exert a controlling influence. In all of our cases with bacteremia, the appearance of serum immunity marked its termination, and in the one case dying after the acquisition of this property, the blood remained sterile. On the other hand, Lord and Persons report two cases in which bacteremia persisted in the presence of acquired serum immunity. Sutliff and Rhoades also observed one case which probably falls into this category. Our cases, in which negative blood cultures were obtained throughout the course of the disease without demonstrable antipneumococcal activity in the serum, imply the existence of other factors operating to prevent the liberation of pneumococci into the blood stream.

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⁵ In a study which has just been published (21), Winkler and Finland found mouse protective action late in the disease in the serum of a number of patients with Type III and higher type (newly classified) pneumonias. Also Finland and Winkler failed to find immune substances in a considerable percentage of Type III and Type VIII pneumonias recovering from the disease (22).

The objection that our methods may not be sufficiently sensitive to detect small concentrations of these immune bodies in the blood cannot be answered directly, but our finding (described in Paper I) of bacteremia in the presence of minimal concentrations of natural immune substances is pertinent in this connection.

The question of the state of functional activity of the phagocytic cells of the body is of importance especially in those instances where the humoral immune substances apparently fail to exert the effects which might logically be expected from their presence. Tests on the phagocytic activity of the circulating leukocytes have failed to disclose any significant disturbance of this function (11) (12) (13). We have substituted leukocytes obtained from patients in different stages of lobar pneumonia, for normal leukocytes in pneumococcidal tests and found them to be equally effective in bringing about the destruction of virulent pneumococci. While these findings do not preclude the occurrence of a diminished antipneumococcal activity of the leukocytes under particular conditions, they do suggest that this function is a relatively stable one. Concerning the activities of the fixed tissue cells we have no information.

Certain findings in studies on the mechanism of recovery from experimental pneumococcus infection are of much interest here. Cecil and Blake (14) failed to detect the presence of mouse protection activity in the serum of monkeys recovering from experimental lobar pneumonia, although these monkeys had acquired well marked immunity as shown by their resistance to re-infection. Cecil and Steffen (15) found that monkeys could be rendered resistant to pneumococcus infection by means of vaccination with killed pneumococci, but they were unable to detect mouse-protective activity in the serum of such immune animals. In a study of repeated attacks of experimental lobar pneumonia in dogs (16) we found marked variations in different animals with respect to the occurrence of detectable humoral immunity. Some dogs showed considerable concentrations of specific immune substances following an attack of the disease; others showed none. Yet these latter animals when tested by re-infection were fully as resistant as the dogs exhibiting acquired humoral immunity. On the other hand, cats recovering from experimental pneumococcus infection regularly developed a relatively high degree of antipneumococcal activity in their blood (17). That the antipneumococcal response of human beings resembles more nearly that of the cat than the other animal species, is born out by clinical findings and the observations of Barach (18) in vaccination of human subjects with pneumococcus vaccine. A high percentage of such vaccinated individuals, patients with lobar pneumonia as well as others without infection of the respiratory tract, showed the appearance of mouse protective properties in the serum within four to six days.

Do the variations in the elaboration of antipneumococcal substances observed in the different animal species or in different individuals of a single

species represent differing responses of the same reaction, or is there another mechanism common to all these species upon which the body depends principally for its recovery from localized pneumococcus infection? In our earlier study of this subject (1), we assigned more importance to the part played by humoral immune properties in recovery from lobar pneumonia than these later and more extensive observations would warrant. Further evidence suggesting that circulating immune substances may, under certain conditions, play a subordinate rôle in this process, has been supplied by several cases of pneumonia in whom we have observed clearing of the pneumonic lesion in one area while extension was taking place elsewhere in the lung. An instance of this kind, pictured in Chart II of Paper I (4), showed progressive resolution of the initially consolidated right lower lobe during the period that spread to the right upper lobe was occurring.[c] A second patient has recently come under our observation in whom early and rapid disappearance of the first consolidated area of the right lower lobe (the lateral inferior segment) occurred while involvement of the remainder of the lobe was taking place. Case 4, Table I, showed this phenomenon on the day preceding crisis. In the study of experimental lobar pneumonia in the dog (20) we have noted frequently clearing of the lesion and spread of the lesion occurring simultaneously in different parts of the lung field. Additional evidence of what might be termed local or regional recovery is provided by our not uncommon finding at autopsy of beginning resolution in certain areas of the consolidated lung in patients without detectable circulatory immune substances. Microscopic examination of such regions shows pneumococci to be few or absent, while in neighboring areas which are still intensely consolidated, microorganisms are usually abundant. Although any proposed explanation of such changes is at present entirely speculative, it would seem to us that the supposition of acquired antipneumococcal immunity by the local tissue cells is not inconsistent with the phenomena observed. It is also conceivable that a widespread specific reaction of the pulmonary fixed tissue cells may play an important part in the mechanism of recovery from lobar pneumonia. This conception would not exclude the participation of immune substances in the process.

SUMMARY

Observations relating the appearance of acquired immune substances to the spread of the disease, blood invasion and recovery were carried out in thirty cases of pneumococcus lobar pneumonia. Acquired antipneumococcal immune properties were detected in the serum in all of the patients recovering and in only one of the six fatal cases. However, the appearance of this immune reaction was not closely related to the onset of recovery. In many instances it was found two or three days beforehand or not until

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[c] X-rays of this patient's lungs are presented in another communication (19).

a day or so afterwards.   While in the majority of the patients there was no increase in the extent of the lesion, as determined by serial x-rays, after the appearance of the newly acquired immune bodies, a further spread of the process did occur in six cases.   Bacteremia ceased either before or coincident with the occurrence of demonstrable humoral immunity.   However, the blood remained sterile throughout the course of the disease in several cases, showing neither natural nor acquired immunity.   Furthermore, the pulmonary lesion remained static for a number of days in some cases showing a similar lack of antipneumococcal activity in their blood serum.

These findings suggest that either the body brings into play more than one mechanism for restraining the spread of the pneumonic process, localizing the invading microorganism within the lesion and eventually terminating the infection, or that the elaboration of humoral immune substances represents only one phase of a specific reaction against the pneumococcus.

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THE RELATION OF CIRCULATING ANTIPNEUMOCOCCAL IMMUNE SUBSTANCES TO THE COURSE OF LOBAR PNEUMONIA

III. INJECTED IMMUNE SUBSTANCES (ANTIPNEUMO-COCCUS SERUM, TYPES I AND II)

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In the consideration of this phase of the subject more attention will be given to certain objective changes occurring after the introduction of immune serum than to mortality. Our series of cases is far too small to permit any conclusions concerning the influence of serum therapy on the outcome of the disease. Mortality percentages will be taken into account in the discussion of the reports of other workers. We wish to present the data which we have secured from a study of the effect of immune serum, chiefly Type I, on the spread of the pneumonic lesion, bacteremia, the duration of the disease and resolution of the consolidated lung.

MATERIALS AND METHODS

The therapeutic antipneumococcus serum, Type I, was obtained from two different sources; one, the New York State Board of Health Laboratories,[1] the other, Lederle and Co. Both sera were of high potency; the concentrated antibody solution, Types I and II (Felton's Method) was secured from Lederle and Co. The dosage of unconcentrated serum employed was that recommended by Cole (1); namely 100 cc. every eight hours until the temperature fell and remained below 39° C. In the administration of the antibody solution we were guided by the recommendation of Cecil and Sutliff (2) that 80,000 to 100,000 units be given within the first twenty-four hours of treatment. The dosage thereafter depended on the changes in the temperature, pulse and respiration. If no improvement was manifest, the dosage was continued for another twenty-four

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[1] Through the kindness of Dr. A. B. Wadsworth we were supplied with serum free of charge for the part of the study carried on at the Peiping Union Medical College.

hours. With improvement, the amount of antibody solution was reduced, and usually discontinued during the ensuing twenty-four or forty-eight hours.

Pneumococcidal tests were carried out exactly as in the preceding section on the study of acquired immune substances. An x-ray was taken, in so far as possible, immediately before the first dose of serum and thereafter at twenty-four hour intervals.

Clinical cases

The data on which the present study is based were secured from observations on twenty-nine cases of lobar pneumonia treated with serum. Of these, all except three were caused by Pneumococcus Type I. The observations on ten patients of the series were carried out at the Peiping Union Medical College and the Hospital of the Rockefeller Institute. The remaining nineteen were studied in the University of Chicago Clinics. A summary of all the findings pertinent to this presentation is shown in Table I.

Occurrence of pneumococcidal-promoting properties in the serum following injection of immune serum

In only one case of the twenty-nine did the patients' serum show any acquired immune activity before the institution of serum therapy. (Table I, Case 25, L. W. P.) After the first twenty-four hours of serum therapy, well marked pneumococcidal-promoting activity was demonstrable in the blood of every patient. The titer of the serum was frequently higher than that commonly observed in patients recovering spontaneously from the disease. With the further administration of the immune serum, the antipneumococcal activity of the patients' serum was maintained or increased. However, following discontinuance of serum therapy there was often a sharp decline in the pneumococcidal-promoting effect of the serum and not infrequently it completely disappeared from the patients' blood within a relatively short time (Chart II). More frequently a gradual diminution in the pneumococcidal-promoting activity occurred over a period of several weeks. As might be expected there was no definite relationship noted between the amount of immune serum given and the degree of pneumococcidal-promoting activity detectable in the patients serum even when rate of administration and stage of the disease were taken into account (Tables I and II).

Effect of immune serum on the spread of the pulmonary lesion

Definite spread of the pulmonary process was detected in only two cases subsequent to the first twenty-four hours of serum therapy. One patient, Number 1 (J. T. A.) (see Table I), showed fresh involvement of an

TABLE I

Relation of injected immune substances to course of disease

Case number	Type	Day of disease immune serum treatment begun	Amount of immune serum injected — In 1st 24 hours	Total within 48 hours	Titer of pneumococcidal-promoting activity of patient's serum — Before immune serum	24 hours after beginning of immune serum	Spread of lesion after 24 hours of serum	Blood invasion — Before serum	After serum	Outcome. Day of beginning recovery or death
1. (J. T. A.)	I	2d	300 cc.	600 cc.	0	1:640	+	0	0	R. 4th day
2. (G. B.)	I	5th	40,000 u.	same	10^{-3}*	10^{-3}*	0	0	0	R. 6th day
3. (M. E.)	I	6th	60,000 u.	same	0	1:320	0	0	0	R. 6th day
4. (G. F.)	II	2d	80,000 u.	100,000 u.	0	1:320	0	+	—	R. 4th day
5. (G. G.)	I	7th	100,000 u.	same	0	1:2560	0	0	0	R. 7th day
6. (G. H.)	I	3d	80,000 u.	same	0	1:2560	0	0	—	R. 4th day
7. (R. H.)	I	3d	110,000 u.	130,000 u.	0	1:1280	0	0	—	R. 5th day
8. (J. L.)	I	4th	300 cc.	900 cc.	0	1:320	0	+	0	R. 7th day
9. (E. M.)	I	1st	80,000 u.	same	0	1:5120	0	+	0	R. 2d day
10. (A. M.)	I	3d	300 cc.	550 cc.	0	1:640	0	0	0	R. 6th day
11. (A. M.)	II	1st	80,000 u.	177,000 u.	0	1:320	0	+	0	R. 6th day
12. (W. H. Mc)	I	7th	300 cc.	same	0	1:1280	0	0	0	R. 8th day
13. (L. B. M.)	I	4th	60,000 u.	120,000 u.	0	1:1280	0	+	0	R. 6th day
14. (R. O.)	I	1st	100 cc.	100 cc.	0	1:40 (After only 50 cc. ser.)	+	0	0	R. 4th day
15. (A. S.)	I	5th	80,000 u.	120,000 u.	0	1:2560	0	0	0	R. 7th day
16. (F. S.)	II	1st	80,000 u.	same	0	1:1280	0	0	0	R. 2d day
17. (M. T.)	I	3d	50,000 u.	70,000 u.	0	1:80	0	+	0	R. 5th day
18. (T. W.)	I	3d	70,000 u.	150,000 u.	0	1:320	?	+	0	R. 7th day
19. (E. Z.)	I	5th	300 cc.	600 cc.	0	1:1280			(Later = +)	D. 12th day

TABLE I (*continued*)

Case number	Type	Day of disease immune serum treatment begun	Amount of immune serum injected†		Titer of pneumococcidal-promoting activity of patient's serum		Spread of lesion after 24 hours of serum	Blood invasion		Outcome. Day of beginning recovery or death
			In 1st 24 hours	Total within 48 hours	Before immune serum	24 hours after beginning of immune serum		Before serum	After serum	
20. (F. P. R.)	I	4th	300 cc.	700 cc.	0**	1:640	0	0	—	R. 7th day
21. (K. R. R.)	I	5th	150 cc.	730 cc.	0	1:320	—	—	—	R. 12th day
22. (H. Y. T.)	I	5th	300 cc.	500 cc.	0	1:320	0	0	—	R. 6th day
23. (H. S.)	I	4th	300 cc.	900 cc.	0	1:80	?	+	0	R. 9th day
24. (B. M.)	I	3d	300 cc.	500 cc.	0	1:320	0	0	—	R. 4th day
25. (L. W. P.)	I	11th	300 cc.	same	1:10	1:640	0	0	—	R. 11th day
26. (F. M. Y.)	I	5th	250 cc.	same	0	1:1280	0	0	—	R. 6th day
27. (L. Y.)	I	12th	200 cc.	same	0	1:160	0	0	—	R. 12th day
28. (L. K. Y.)	I	6th	300 cc.	400 cc.	0	1:80	0	0	—	R. 8th day
29. (W. F.)	I	2d	400 cc.	same	0	1:1280	0	0	—	R. 2d day

† In column under heading "Amount of immune serum injected" the unconcentrated serum is designated in cc. while the concentrated antibody solution (Felton) is indicated in units.

The figures in the column under "Titer of pneumococcidal-promoting activity of patient's serum" indicate that dilution of the patient's serum which when added to 0.2 cc. of fresh rabbit serum + rabbit leukocytes (concentration of W. B. C. as before) is capable of destroying 10^{-6} of the standard suspension of pneumococci.

* In this case pneumococcidal tests were made only on the whole serum. The increase in the number of organisms killed, from 10^{-5} (of the standard suspension) before serum to 10^{-3} afterwards indicates a decided increase in the pneumococcidal-promoting power of the serum.

** Two days earlier the serum of this patient had shown a pneumococcidal-promoting titer of 1:20 as well as mouse protection action.

TABLE II

Relation of pneumococcidal-promoting titer of patient's serum to length of disease course in cases treated within the first three days

Case number and type	At beginning of serum treatment		Day of disease on which immune serum begun	Day of recovery	Titer of pneumococcidal-promoting activity of patient's serum in days following the first 24 hours of serum treatment*					
	Extent of lung area involved**	Bacteremia			1	2	3	4	5	6
11. (A. M.) II	L1/4	0	1st	6th	1:320	—	1:160	1:320	1:40	
14. (R. O.) I	L1/3	0	1st	4th	1:40	1:2560	1:640			
16. (F. S.) II	R1/4	0	1st	2d	1:1280					
9. (E. M.) I	R1/2	+	1st	2d	1:5120					
1. (J. T. A.) I	L1/8	0	2d	4th	1:640	1:320				
4. (G. F.) II	R1/6	0	2d	4th	1:320	1:320				
29. (W. F.) I	R1/2+L1/2	0	2d	2d	1:1280					
6. (G. H.) I	R1/8	0	3d	4th	1:2560					
7. (R. H.)	R1/8	+	3d	5th	1:1280	1:2560	1:5120			
10. (A. M.) I	L1/3	0	3d	6th	1:640	1:2560				
17. (M. T.) I	L1/4	+	3d	5th	1:80	1:40				
18. (T. W.) I	L1/3	+	3d	7th	1:320	1:320	1:160	1:160		
13. (L. R. M.) I	L2/3	+	3d	4th	1:320					

* The daily serum titer up to and including the day of beginning recovery is recorded.

** The figures L1/4, R1/2, etc. indicate the approximate area of the left and right lung fields occupied by the x-ray shadow.

adjacent lobe in the presence of well marked pneumococcidal-promoting activity of the serum. Extension of the process occurred for one day only when the disease was cut short by crisis on the 4th day. The other case, Number 14 (R. O.), required desensitization and obtained considerably less than the usual amount of serum in the first twenty-four hours. In two additional patients there was a questionable spread of the lesion after the first day's treatment. The x-rays showed increasing density of already involved areas which might well be interpreted as indicating more effective localization of the disease process. Thus, while it is not possible to make any comparison with untreated cases on a statistical basis, it does seem that in this series of patients, spread of the disease occurred less often than is observed in cases of Types I and II lobar pneumonia that are not treated with serum.

Effect of immune serum on bacteremia

Positive blood cultures were obtained in six cases before the beginning of serum treatment. The highest colony count was 9 per cc. of blood. In every case the cultures became negative within twenty-four hours and remained so with one exception. This patient, Number 19 (E. Z.), an old woman 77 years of age, had apparently recovered from her disease when, after four days without fever, she rapidly became comatose and died. On the day of death, the blood was found to contain pneumococci and to be without pneumococcidal-promoting properties. Thus, there would appear to be a direct relationship between the presence of an excess of circulating pneumococcal immune substances and the localization of pneumococci in the lung lesion. However, in none of our cases were there more than a few colonies of pneumococci found in blood culture, a fact which necessitates caution in drawing inferences from this study.

The relation of treatment by immune serum to the length of the course of the disease

The duration of the disease in the serum treated cases was found to depend largely on the time at which the serum was begun (Tables I and II). Six of the seven patients treated in the first forty-eight hours of the disease recovered within four days [2] (Table II). In the seventh case, caused by pneumococcus Type II, the disease continued until the sixth day, although treatment was begun on the first day of his illness. Of three Type I cases treated within 30 hours of onset two (Cases 9 and 29) recovered promptly. The third (Case 24) receiving an inadequate amount of serum because of sensitivity to horse protein did not recover until the 4th day. One of the two Type II patients treated in the first 24 hours showed an

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[2] The day on which the final decline in temperature, pulse and respiration began is taken as the day of recovery.

immediate response. The other was the case just mentioned. Of six patients treated for the first time on the third day of the disease, four recovered within five days, the other two on the 6th and 7th days respectively. When immune serum was given after the third day, it appeared to have little effect on the duration of the disease (Table I) and Chart I and II.
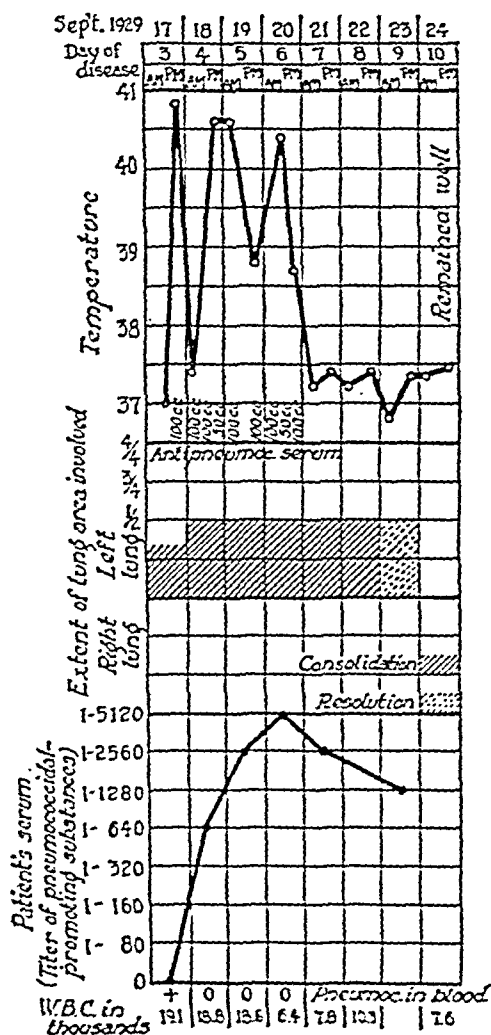
CHART I. CASE 10 (A. M.). LOBAR PNEUMONIA. PNEUMOCOCCUS TYPE I ISOLATED FROM THE LUNG

The relationship of the titer of the pneumococcidal-promoting activity of the serum to the continuance of the disease is difficult to evaluate from the data given in Table II. In certain cases the disease persisted from three

to five days in the presence of a concentration of circulating immune substances as high or higher than that shown by other cases in which recovery occurred within twenty-four to forty-eight hours after the first dose of
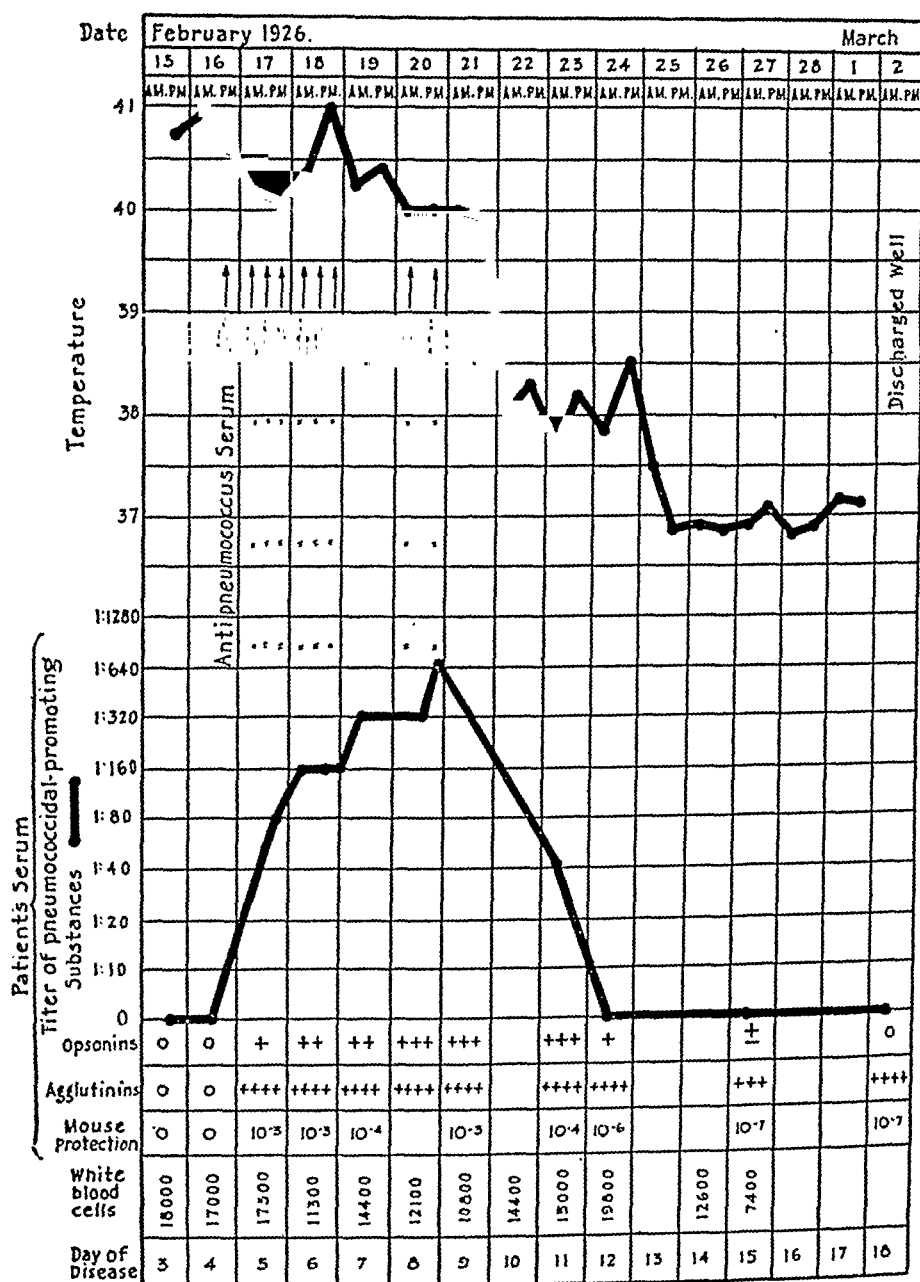
CHART II. CASE 23 (H. S.). LOBAR PNEUMONIA. PNEUMOCOCCUS TYPE I ISOLATED FROM THE BLOOD. SERUM TREATMENT BEGUN ON THE 4TH DAY OF THE DISEASE.

serum. It is true that the highest initial titers of serum activity were found in those patients recovering within twenty-four hours after the beginning of treatment, but that only moderate concentrations of immune bodies may bring about the same effect was shown by Case 13 (I. B. M.), Table II.

There was no observed relationship between the extent of pulmonary involvement present at the time serum was begun and the duration of the
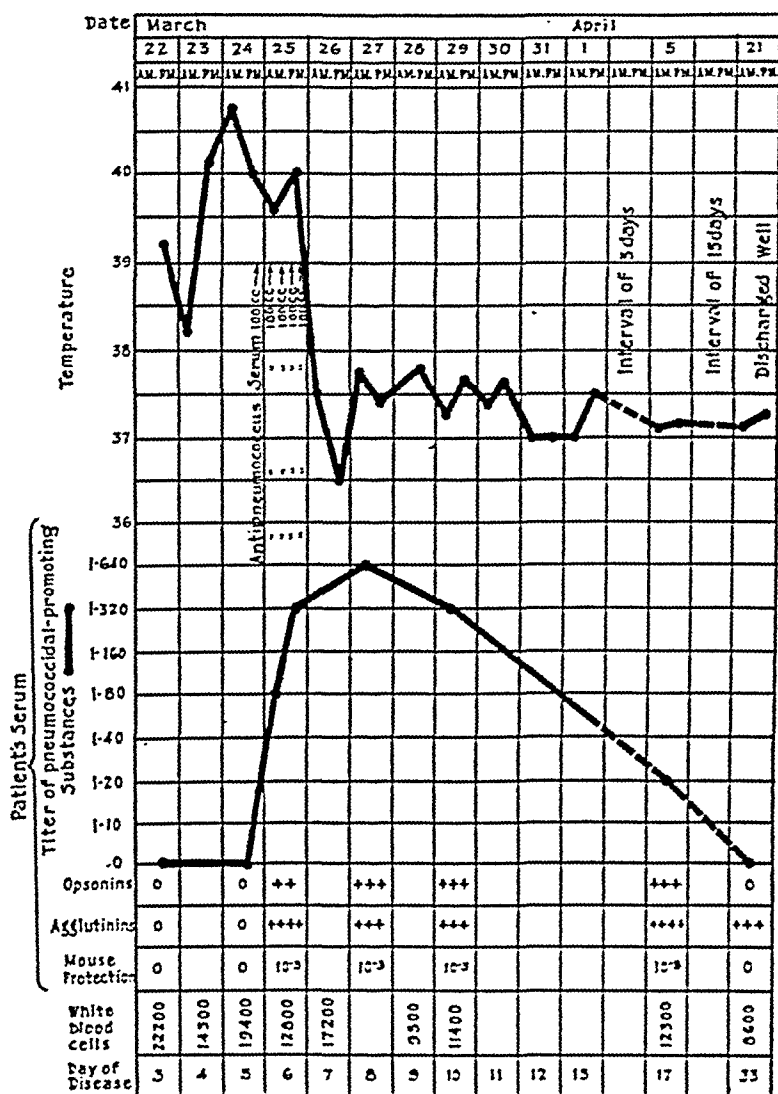
CHART III. CASE 22 (H. Y. T.). LOBAR PNEUMONIA. PNEUMOCOCCUS TYPE I ISOLATED FROM THE SPUTUM. SERUM TREATMENT BEGUN ON THE 5TH DAY OF THE DISEASE.

disease thereafter. In fact, the two patients, Cases 29 and 13, showing the most extensive lesions, recovered within twenty-four hours after the initiation of immune serum therapy.

Effect on resolution

The injection of immune serum appeared to have no effect on hastening the onset of resolution except in two cases in which treatment was begun on the first day of the disease. These two patients, Cases 9 and 16 (Tables I and II) both had a crisis the day following the initiation of treatment. One showed definite signs of beginning resolution on the 3d day after the onset of the disease and the other on the 4th day. A third case, Number 11, Pneumococcus Type II, treated on the first day of his illness, did not recover until the 6th day when resolution was first noted. Patients treated on the second day or later showed resolution occurring from the 6th to the 9th days after onset, even though the course of the disease was shorter than usual.

Comparative observations in the several manifestations of humoral immunity in the patient's serum

In certain cases, tests for the opsonic, agglutinative and mouse protective, as well as the pneumococcidal-promoting activity, were made on the same sample of serum. The results of such observations are illustrated in Charts II and III. Before the injections of immune serum and for some days afterwards, there was found to be a close parallelism between these different reactions. However, with the diminution of pneumococcidal-promoting potency of the patient's serum, there occurred in most of the cases tested, a curious disparity between the agglutinating properties of the serum and its other antipneumococcal manifestations. As will be noted in the accompanying charts, final tests showed little or no pneumococcidal-promoting, mouse protective or opsonic activity, but a marked and practically undiminished agglutination reaction. We made no further studies as to the nature of this phenomenon and have no explanation to offer.

Mouse protective action was found to be a somewhat more delicate test for antipneumococcal properties of the serum than was its pneumococcidal-promoting action as tested here. However, the latter is considerably more sensitive to quantitative estimation and gives more regular results.

DISCUSSION

The majority of reports on the use of antipneumococcus serum in the treatment of lobar pneumonia have dealt principally with its effect on mortality. A few studies have included observations on those objective changes occurring during the course of the disease which may be attributed to the introduction of the immune serum. The inhibiting action of antipneumococcus serum on the spread of the pneumonic lesion, first reported by Cole (3) in Type I pneumonia has been confirmed by later workers. Finland and Sutliff (4) found likewise that in Type II pneumococcus pneumonias treated with large doses of Felton's concentrated antibody

solution, extension of the lesion ceased in every case. The data for these observations were secured both from physical examinations and x-ray, although details as to the frequency of x-ray examinations, the extent and location of the shadow, etc., are not given. Our observations which are based on daily x-ray examinations agree substantially with those of the afore mentioned workers. The one exception among 29 cases, that of a spread to a new lobe occurring in an early well treated patient subsequent to the first twenty-four hours of therapy, represents the occasional failure of even a high concentration of circulating antipneumococcus immune bodies to check the extension of the lesion. However, growth of the pathological process ceased within forty-eight hours and the patient recovered on the fourth day of the disease.

Another and even more significant effect of antipneumococcus serum which has been found to occur constantly, is its power to control bacteremia. In Cole's report (5), of 431 cases of Type I lobar pneumonia treated with serum, among which 140 showed blood invasion before the initiation of specific therapy, the blood became sterile in all but three instances. These three patients showed initial colony counts of several hundred pneumococci per cc. of blood. Even in cases with intense bacteremia the injection of immune serum in large doses was found to produce a marked reduction in the number of micro-organisms in the blood or at times, their complete disappearance. Sutliff and Finland (4) (6), and Cecil and Plummer (7), report analogous results in both Types I and II lobar pneumonia treated with concentrated antibody solution (Felton).

As to whether the course of the disease is shortened by the administration of immune serum, there is less unanimity of opinion. Locke (8) found no difference between serum treated and control patients in the duration of the disease, although he notes that in patients treated within the first three or four days of the disease the subsequent temperature curve was lower than in the untreated cases. On the other hand, Cecil and Sutliff (2) found in a larger series of cases, including both Types I and II lobar pneumonia, that the mean temperature curve of the serum treated cases was about two days shorter than that of the control untreated patients. Likewise, Armstrong and Johnson (9) observed that the course of the disease in the cases treated by Type I antiserum was shortened two and one-half days and the cases treated by Type II antiserum one and one-half days as compared with an equal number of controls. Sutliff and Finland (6) and Finland and Sutliff (4) found that in cases treated on or before the fourth day, recovery occurred one to two days earlier than in the untreated patients. Our observations coincide with those of the last named authors although in individual cases there was no evidence that the disease was abbreviated when treatment was initiated later than seventy-two hours after onset. The most striking effect of serum is seen in Type I patients treated

within the first twenty-four hours of the disease. Recovery usually occurs promptly.

Granted that the duration of the disease is in general shortened by the administration of immune serum and the evidence available supports this inference, there still remains a number of instances in which the disease persists actively for days despite a high concentration of injected specific immune substances in the blood. This condition which we have observed repeatedly presents a problem of great interest in relation to the mechanism of the action of immune serum. The data obtained on such cases fail to reveal any unusual bodily state which might interfere with the full action of serum. Case 10 (A. M.) Chart I for example, showed an x-ray shadow covering only one-third of his left lower lung field and a bacteremia of 1 colony per cc. of blood at the time of beginning serum treatment on the 3d day of the disease. After twenty-four hours of serum treatment (300 cc.) the blood culture was sterile, and the process, which now occupied only half the lung field, stopped spreading. The concentration of immune substances in his blood increased to a point considerably beyond that shown by the average patient recovering spontaneously, but the symptoms and signs of the disease persisted until the night of the 6th day. In this patient the number of white blood cells was rather low during the last two days of his illness, but a well marked leukocytosis was present before that time. This patient in a second attack of lobar pneumonia, due to pneumococcus Type II (Case 11) showed the same phenomenon of a prolonged disease even though antibody solution was begun on the first day of the disease and a fair concentration of immune substances was maintained during the six days of his illness. The leukocytes did not rise above a high normal at any time. It is very doubtful, however, whether the lack of a high white count had anything to do with the prolonged course of the disease, since patients exhibiting this same condition often have had marked leukocytosis.[3]

Another patient (Case 20, Table I and Chart II) grew steadily worse symptomatically during the first three days of intensive treatment, becoming comatose on the 7th day of the disease. While the serum checked

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[3] In a previous study of the pneumococcidal action of mixtures of rabbit serum and leukocytes containing specific antipneumococcus serum, it was found that the presence of a small amount of fresh normal serum was essential to the reaction. This activating effect of the normal serum could be abolished by heating it at 56° C. for one-half hour or by ageing the serum. In order to determine whether this property of the normal serum is diminished during pneumococcus infection, pneumococcidal tests were made in which serum secured from pneumonia patients at various stages in the disease was substituted for normal rabbit serum. It was found that the activating effect of the serum in lobar pneumonia was not impaired to any appreciable degree (22). Hence, the apparent failure of the immune serum to exert its expected action in the cases described above is not to be attributed to such a deficiency of the circulating blood fluid. The state of the functional activity of the leukocytes in lobar pneumonia has been discussed in the preceding paper.

blood invasion and the lesion remained confined to the two lower and right upper lobes, no antitoxin action could be ascribed to the immune serum. The possible implications of these observations will be discussed at the end of the paper in connection with the general significance of immune bodies.

Wadsworth (10) has summarized the reports on 853 cases of Type I pneumonia treated with whole antipneumococcus serum which showed a mortality for the group of 12.4 per cent. Including an additional 200 cases reported by Cole (5) which were not included in Wadsworth's group, the mortality on more than 1,000 serum treated cases would not be over 12 per cent. Cole's figures on 431 cases treated at the Hospital of the Rockefeller Institute gave a mortality of 10.2 per cent. Most reports on the results of treatment of Type I pneumonia with concentrated antibody solution (Felton) have shown a considerably higher mortality. Cecil and Plummer's (11) series of 239 cases showed a mortality of 20.1 per cent. Park, Bullowa and Rosenblüth (12) report a death rate of 17 per cent in a series of 109 cases. In each of these two series the control cases showed a higher mortality than that given by Wadsworth, the result being that the relative reduction of mortality in patients treated by these two kinds of immune serum is not very different. However, a somewhat more favorable report on the use of Felton's antibody solution has been recently made by Heffron and Anderson (21) who observed a mortality of only 10.6 per cent among 188 Type I cases treated within the first four days of the disease, as compared with 25.9 per cent mortality on the control series. Results of treatment of pneumococcus Type II pneumonia with antibody solution have been for the most part less favorable. In Cecil and Plummer's (7) series of 252 serum treated cases, there was a mortality of 40.5 per cent as compared with 45 per cent in the control cases. Park and his co-workers (12) reported 56 cases with a mortality of 23 per cent as opposed to 30 per cent in the untreated cases. On the other hand Finland and Sutliff (4) treated a series of 46 cases with a mortality of 20 per cent as contrasted with a mortality of 40 per cent in 81 untreated cases. These latter authors used considerably larger doses of antibody solution than had been previously employed. These results obtained by a number of workers show without any doubt that the administration of antipneumococcus immune serum of high potency in adequate dosage brings about reduction in mortality in Type I pneumococcus lobar pneumonia and to a less, but definite degree in pneumonia caused by Type II. When the serum is administered within the first three days of the disease the death rate is further diminished. In several large series the mortality per cent of patients treated in the first 72 hours of the disease is half that of the group mortality.

It has been found by all observers that patients showing an initial bacteremia respond much less well to the serum. The presence of blood invasion as Bloomfield (13) pointed out indicates that the body's defense mechanism has already begun to break down and hence the therapeutic

within the first twenty-four hours of the disease. Recovery usually occurs promptly.

Granted that the duration of the disease is in general shortened by the administration of immune serum and the evidence available supports this inference, there still remains a number of instances in which the disease persists actively for days despite a high concentration of injected specific immune substances in the blood. This condition which we have observed repeatedly presents a problem of great interest in relation to the mechanism of the action of immune serum. The data obtained on such cases fail to reveal any unusual bodily state which might interfere with the full action of serum. Case 10 (A. M.) Chart I for example, showed an x-ray shadow covering only one-third of his left lower lung field and a bacteremia of 1 colony per cc. of blood at the time of beginning serum treatment on the 3d day of the disease. After twenty-four hours of serum treatment (300 cc.) the blood culture was sterile, and the process, which now occupied only half the lung field, stopped spreading. The concentration of immune substances in his blood increased to a point considerably beyond that shown by the average patient recovering spontaneously, but the symptoms and signs of the disease persisted until the night of the 6th day. In this patient the number of white blood cells was rather low during the last two days of his illness, but a well marked leukocytosis was present before that time. This patient in a second attack of lobar pneumonia, due to pneumococcus Type II (Case 11) showed the same phenomenon of a prolonged disease even though antibody solution was begun on the first day of the disease and a fair concentration of immune substances was maintained during the six days of his illness. The leukocytes did not rise above a high normal at any time. It is very doubtful, however, whether the lack of a high white count had anything to do with the prolonged course of the disease, since patients exhibiting this·same condition often have had marked leukocytosis.[3]

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serum which, as we know, depends for its action on the co-operation of the tissues, could not be expected to exert its maximum beneficial effect in such cases. It is of much interest to inquire in this connection into the cause of death of serum treated patients in whom the blood has become sterile under the influence of serum and the spread of the lesion arrested. An analysis of the fatal cases such as is given by Cole (5), Cecil and Plummer (11), Finland and Sutliff (4) shows that the majority of patients dying after treatment with immune serum have some severe complicating condition either present before or developing during the pneumonic process. A certain small percentage of patients, however, in whom treatment was begun moderately early in the course of the disease and continued adequately, have gone on to a fatal termination without complications and with a well localized and relatively circumscribed pulmonary infection.

In attempting to elucidate this problem, it would be important to know how frequently death occurs in patients who were not treated by serum and who showed involvement of single lobes with sterile blood cultures and no complications. Unfortunately, little has been written on the subject. However, two valuable studies on Types I and II pneumonia (as yet unpublished) have recently been made by Sutliff and Finland [4] which include pertinent data. Sutliff found that of 19 Type I pneumonia patients dying with only a single lobe consolidated, six showed sterile blood cultures during life and three of them died without complications. Finland had a series of 24 fatal Type II cases with the pneumonic process confined to one lobe, five of which died without detectable bacteremia. His data did not include the presence of complications. Both these investigators have made similar observations on smaller groups of patients treated by serum. Of Sutliff's nine cases dying with a single lobe involved, seven showed negative blood cultures and five of them had no complications. It is true that in this series certain cases received small amounts of serum and others were in the very old age group, but the significant fact is that they died with a well localized lesion of one lobe only. In Finland's series, of six serum treated cases dying with the lesion confined to a single lobe, three showed an absence of bacteremia.

How then are all these various observations on the use of immune serum to be explained and coordinated? Why is it that the injection of an adequate dose of antipneumococcus serum within the first 24 to 30 hours of the infection commonly brings about prompt recovery, while beginning the treatment later in the disease results at best in shortening its course by a day or so, or if serum is started after the third day, in the persistence of the process until time of usual spontaneous recovery? How are we to account for the striking action of the immune serum in checking bacteremia and limiting the spread of the lesion, in relation to its apparent inability in

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[4] We are greatly indebted to Drs. Sutliff and Finland for their courtesy in sending us this material and permitting us to use it.

many instances to bring about a cessation of the activity of the disease process before the period of its natural termination? And why do some patients die with only a single lobe involved and no blood invasion? While unequivocal answers to these questions are not possible without much more data than is at present available. we would like to propose a viewpoint based on inferences derived from our work and that of others.

It is well known that mixtures of fresh normal leukocytes and highly potent antipneumococcus serum with a small added quantity of fresh normal serum are capable of exerting marked pneumococcidal action under conditions providing maximum contact between the pneumococci and the leukocytes. The early developing intra-alveolar exudate in lobar pneumonia consists of edema fluid into which pour an increasing concentration of young active polymorphonuclear leukocytes (14). By means of the respiratory movements and the increasing accumulation of fluid and cells within the alveoli, the suspended pneumococci are brought into intimate contact with the cellular elements. While the natural antibodies are active and produce a certain degree of opsonization, as we were able to observe in experimental lobar pneumonia in the dog and early lesions in human beings (15), they are not present in sufficient concentration to check the expansion of the lesion. If, however, at this stage, a high concentration of immune substances is produced in the blood stream, the process of phagocytosis and intracellular digestion is so enhanced as to bring about a rapid diminution in the number of invading micro-organisms and the consequent termination of the infection. As the lesion progresses, the alveoli become packed with leukocytes, fibrin is laid down, the whole mass of diseased tissue becomes less mobile and there is a decrease in the richness of the blood supply. In our study of the x-ray changes in lobar pneumonia (16), we found that it usually required about three days for the lesion to reach its maximum density. It is after this stage that the introduction of immune serum is less effective. Various ideas have been put forward in an attempt to explain the phenomenon, chief among which is the lack of penetration of the immune substances into the diseased area because of·obstructed blood supply. The objection to this conception is the lack of evidence of a markedly defective circulation in the well consolidated lesion. If a serious impairment of the blood supply was present, one would expect much more necrosis of the lung tissue than is found in the uncomplicated lesion at autopsy. Furthermore, the toxic manifestations of the disease indicate the constant liberation of injurious substances from the infected area and the presence of circulating specific soluble substance gives evidence that large molecular structures can pass through into the blood stream. It seems to us more likely that the leukocytes of the intra-alveolar exudate gradually lose their functional activity and hence are unable to cooperate with the immune substances after a certain period of time. The active life of the polymorphonuclear leukocyte has been variously estimated from two or three to four

or five days so that unless there is a constant accession of new cells [5] the exudate would contain more and more dead and dying leukocytes as the disease progresses.   Studies by Kredel and Van Sant (17) on the exudate of the lesions in experimental lobar pneumonia in dogs show that the relative number of dead leukocytes increases progressively after 24 to 48 hours. We have some evidence from both perfusion experiments on the lungs of experimental pneumonia as well as on the studies of tissues that the dog maintains a more abundant blood supply in the consolidated lung and shows much less intra-alveolar fibrin than does the human being, hence we should except an even greater proportion of inactive leukocytes in human pneumonic lung.   The increasing immobility of the progressively consolidating lesion may also interfere with the optimum action of the serum and leukocytes, and no doubt, diminished blood supply, such as is apparently present in the stage of gray hepatization, plays a rôle.   If this is the correct interpretation of the effect of immune serum, the action of a high concentration of circulating immune substances in preventing a spread of the lesion is readily understandable since any new implantation of pneumococci presents the same favorable conditions for the action of the antipneumococcal immune bodies and leukocytes as does the initial lesion.

What action, if any, can be ascribed to immune serum in bringing about recovery when administered after the lesion has been well established? Our observations on the occurrence of acquired immune substances presented in Part II of this study provided no evidence that recovery was dependent on or even necessarily associated with the presence of circulating immune bodies.   Further evidence of the same nature is afforded by the numerous instances of serum-treated patients in our series showing a very high concentration of immune substances for many days preceding recovery.   It is true that such patients all recovered, but the initiation of the recovery process cannot be ascribed to the antipneumococcal activity of the serum, except insofar as it inhibited bacteremia and prevented spread of the lesion.   Are these two effects of the immune serum sufficient to account for the lowered mortality of patients treated after the early stage of the disease?   While adequate data for a statistical solution of this question is not available, certain facts relating extent of pulmonary involvement and bacteremia to outcome are well recognized.   It has been long known that with spread of the pneumonic lesion from lobe to lobe the mortality rises rapidly.   In a study of 658 autopsies on lobar pneumonia patients made by Chatard and summarized by Cole (19) 17 per cent of them showed only a single lobe consolidated whereas the two lobe involvements comprised 34 per cent of the total.   Again the prognostic significance of bacteremia has been pointed out repeatedly in recent years.   In a series of 149 cases of

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[5] Loeschke (14) considers that there is a re-accumulation of leukocytes in the consolidated area, but Lauche, (18) and others find no evidence for this.   Our observations would lead us to concur with these latter workers.

Type I, II and III pneumonia studied by Rosenblüth (20), the mortality of patients with bacteremia was 80 per cent, but only 18 per cent in those without blood invasion. Similarly Cecil and Plummer (7) report a mortality of 87 per cent of Type II cases with bacteremia and 8 per cent without bacteremia. Thus, taking these facts together, it seems obvious that limiting the extent of the infection in the lung and preventing the escape of pneumococci into the blood stream would result in a considerable reduction in mortality. It is also possible that the same action of the immune bodies on the pneumococci in the lesion which is so marked in the early stage, goes on at a slower rate in the older process. Again there may be other effects of the immune serum. Cole, Cecil and others have observed a slight but definite lowering of the fever following the administration of serum in adequate dosage which suggests some antitoxic action. If such an effect is exerted it might account for the briefer course of the disease in serum treated patients reported by certain investigators, in that under condition of lessened toxicity the body is enabled to develop its natural mechanism of recovery more rapidly than otherwise. That the antitoxic effect of immune serum plays more than a minor rôle in the therapeutic action seems unlikely in view of those cases studied by Sutliff and Finland which died with only a single lobe involved, a sterile blood and no complications. However, it should be pointed out here that in Cole's larger series of cases, there were no deaths among patients showing analogous conditions of the disease.[c] This may be significant in relation to the therapeutic preparation employed. Cole used whole antipneumococcus serum, while both Sutliff and Finland used concentrated antibody solution. In our study we were unable to detect any difference between the effects of the two preparations.

SUMMARY

A study of twenty-nine cases of pneumococcus lobar pneumonia treated with specific immune serum was made with the purpose of determining the effect of serum therapy on the spread of the pneumonic lesion, bacteremia, the length of the disease and resolution of the consolidated lung. In twenty-six of the patients the disease was due to Pneumococcus Type I, the remaining three to Pneumococcus Type II. Two preparations of immune serum were used; whole serum Type I, with which the majority of the cases were treated and concentrated antibody solution (Felton) Types I and II. The concentration of antipneumococcal immune substances in the patients' serum was determined by testing its pneumococcidal-promoting power. X-rays of the chest were made daily throughout the course of the illness.

Following the first twenty-four hours of serum treatment, extension of

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[c] Dr. Rufus Cole in a personal communication has kindly supplied me with this data supplementing his analysis of fatal serum treated cases to which reference has been made (5).

the pulmonary lesion ceased in all but two instances. · One of these received an inadequate amount of serum in the first twenty-four hours because of necessary desensitization. The other, adequately treated, showed nevertheless, a spread of the process to a new lobe, but recovered on the fourth day of the disease. Bacteremia terminated in every case with the institution of specific therapy. The effect of immune serum on the course of the disease was found to depend largely on the stage of the illness at which treatment was begun. In the majority of cases treated within the first three days after onset, the course of the illness was shortened by a day or two. Six of the seven patients treated in the first forty-eight hours of the disease recovered within four days. Five of these patients received the first dose of serum within thirty hours of the onset; three of them recovered promptly, and two showed signs of beginning resolution on the third and fourth days respectively after the inception of the disease. In no other instances was there any evidence that serum treatment hastened resolution. When serum was begun after the first three days, the disease usually continued actively until the time of its natural termination despite the presence frequently of a very high concentration of antipneumococcal immune substances in the blood. This finding is of especial interest in relation to the mode of action of immune serum in pneumonia. Our observations and those of others suggest that in the early phases of the disease before consolidation has developed to its maximum intensity, the specific antibodies are capable of so affecting the pneumococci in the lesion as to bring about a rapid cessation of the disease process. But after this stage has been reached the chief effect of the injected immune substances is to confine the pneumococci to the pulmonary lesion and to prevent extension of the pathological process, the actual termination of the disease being occasioned by the unknown process of natural recovery. Data in support of these inferences and evidence that immune serum exerts a favorable influence on outcome even when administered after the disease has developed fully, are presented in detail.

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RELATION OF VARIATIONS IN MEAN CORPUSCULAR VOLUME TO NUMBER OF RETICULOCYTES IN PERNICIOUS ANEMIA

THE SIGNIFICANCE OF INCREASED BONE MARROW ACTIVITY IN DETERMINING THE MEAN SIZE OF RED CORPUSCLES

By M. M. WINTROBE

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It is well known that the macrocytosis of the red corpuscles in pernicious anemia, is as a whole inversely proportional to the degree of anemia and that, as the anemia decreases the red cells become smaller. In an earlier report (1) it was pointed out that the correlation between the red cell count and the mean volume of the erythrocytes is very close when the anemia is only moderate in degree but that this correlation is not so definite in the more advanced stages of anemia. It was also noted that the decrease in the size of the red corpuscles which follows liver therapy, may be preceded by a temporary increase in the mean volume of the red cells. The cause of this temporary increase in macrocytosis was not considered in any detail but it was observed that, in two cases in which reticulocyte counts were made, the peak of the reticulocyte curve coincided with the maximum value for mean corpuscular volume.

Fitzhugh and Persons (2) noted a brief initial drop in mean red cell diameter in pernicious anemia during treatment and a temporary increase in size following this. Brugsch (3) observed an increase in the mean volume as well as the mean diameter of the red corpuscles which occurred in six patients during the second and third weeks of treatment. The former authors made no attempt to explain the variations in cell diameter, but Brugsch attributed the changes to a decrease of microcyte formation and an increase of macrocytes. He observed, however, that the maximum reticulocyte counts coincided fairly well with the period of greatest increase in cell size.

The observations of these investigators as well as my own earlier studies, were made at infrequent intervals and consequently do not afford adequate information concerning the variations in the size of the red corpuscles. The present more detailed examinations have therefore been carried out.

OBSERVATIONS

Mean corpuscular volume has been determined at frequent intervals, usually every third or fourth day and in a number of cases daily, during the course of liver therapy in thirty-two cases of pernicious anemia. The methods employed have been described elsewhere (4).

In Figure 1, *A*, variations in mean corpuscular volume such as were frequently observed, are shown. Immediately following the institution of treatment, and sometimes even before therapy was commenced, in a number
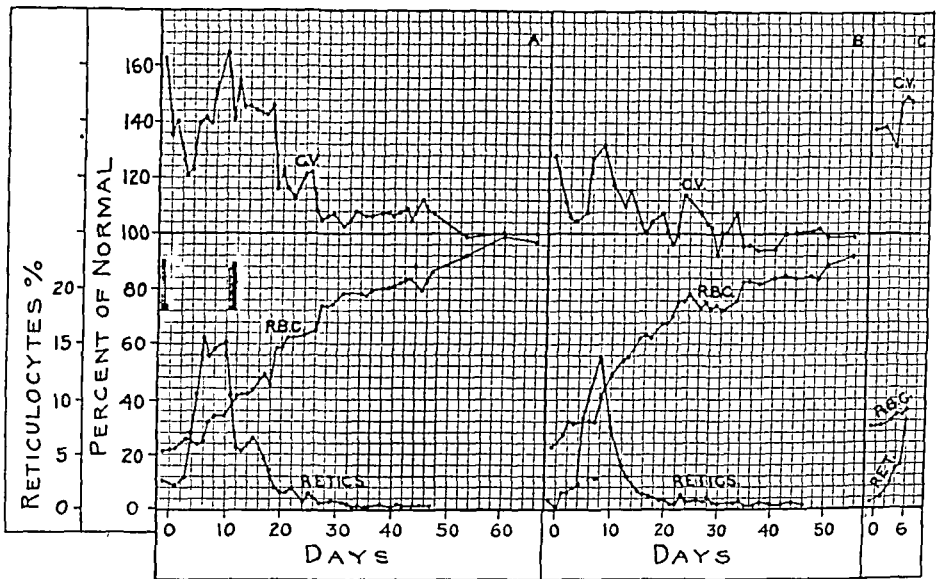
FIG. 1. VARIATIONS IN MEAN VOLUME OF RED CORPUSCLES COMPARED WITH RETICULOCYTE COUNT IN THREE CASES OF PERNICIOUS ANEMIA

The mean corpuscular volume (C.V.) and the red cell count (R.B.C.) are represented as per cent of their respective average normal values. By this method the red cell count and mean corpuscular volume of a hypothetical normal individual would fall on the line at 100 per cent. Reticulocytes are recorded directly. The abscissa records days following the commencement of liver therapy. The columns represent absolute numbers of red corpuscles 6 microns or less in diameter (hatched columns) and absolute numbers of reticulocytes (solid columns). Each division represents 50,000 cells per c. mm.

of cases there was a decrease in the mean volume of the red corpuscles. Within a few days, however, there followed an increase in mean corpuscular volume and the maximum attained was in many instances greater than that observed before treatment was instituted. Following this, the volume gradually decreased until finally, when the red count reached normal, normal values for mean corpuscular volume were usually attained.

To this general type of curve there were, however, many exceptions. In a number of cases the preliminary decrease in mean corpuscular volume was not observed (see Figures 2, *B*, and 3, *A*). The magnitude of the

macrocytosis varied and did not appear to be correlated with the degree of anemia alone, although this no doubt is a very important factor (1). The rapidity with which the macrocytosis decreased also differed somewhat in different cases. However, although the form of the "volume curve" varied, it was observed that in 25 instances (78 per cent) the decrease in the size of the cells following liver therapy, was preceded by a distinct increase in the size of the red blood cells.

Daily corpuscular volume studies and daily reticulocyte counts were carried out in 9 consecutive cases. The observations are shown in Figures 1, 2, and 3.
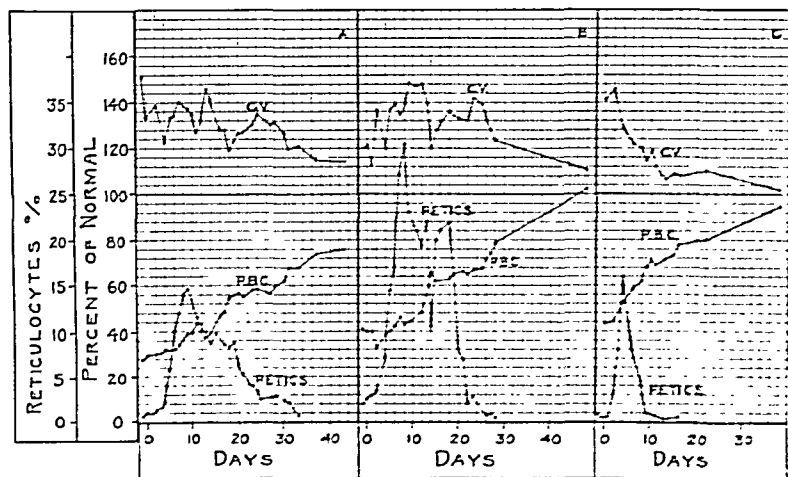
FIG. 2. VARIATIONS IN MEAN VOLUME OF RED CORPUSCLES COMPARED WITH RETICULOCYTE COUNT IN THREE CASES OF PERNICIOUS ANEMIA

Legends same as in Figure 1.

In two instances (Figure 1, A and B) the peak of the increase in corpuscular volume coincided with the peak of the reticulocyte curve. In two cases (Figure 3, B and Figure 2, A) the maximum increase in volume occurred two and four days, respectively, following the observed maximum increase in reticulocytes. In a fifth case (Figure 2, B) a double peak in the reticulocyte curve was associated with a somewhat similar fluctuation in the volume curve. In this case the peaks of the volume curve followed those of the reticulocyte curve in 2 and in 5 days. In another case (Figure 3, A) the peak in the volume curve followed by 8 days that of the reticulocyte curve. Only a few observations could be made on the patient whose blood changes are shown in Figure 1, A.

In only one instance (Figure 2, C) did the peak of the volume curve precede (by two days) that of the reticulocyte curve. In the ninth case

(Figure 3, C) the correlation between the two curves was least evident. It is perhaps noteworthy that the anemia in this case was less marked than in any of the other cases and the reticulocyte response to liver therapy was relatively slight.

In the patients whose blood examinations are shown in Figures 1, A and 3, A, the diameters of the red corpuscles were measured at intervals by means of an ocular micrometer. The absolute numbers of cells 6 microns or less in diameter, are recorded on the charts and compared with the absolute numbers of reticulocytes. These data indicate that the number of microcytes and small poikilocytes showed relatively little change during the time when reticulocytes and corpuscular volume increased.
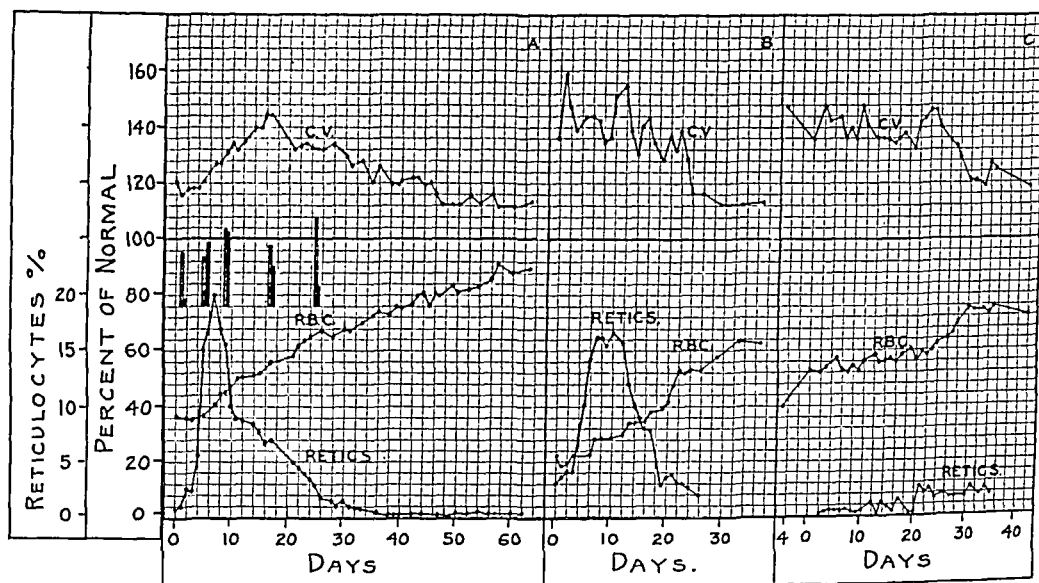
FIG. 3. VARIATIONS IN MEAN VOLUME OF RED CORPUSCLES COMPARED WITH RETICULOCYTE COUNT IN THREE CASES OF PERNICIOUS ANEMIA
Legends same as in Figure 1.

Icterus index determinations were carried out in each of the nine patients observed daily. In two patients there was a decrease in the icterus index (from 18 to 10, and from 11 to 7.5, patients of Figure 2, B and Figure 3, A, respectively) during the first 5 days following the institution of treatment, and in one patient (Figure 1, B) the icterus index decreased from 7.5 to 5 units at the time of the maximum increase in corpuscular volume; during the remaining period of observation in these patients, and during the whole period of observation in the other 6 cases, no consistent tendency of the icterus index either to rise or fall, was noted.

DISCUSSION

The data presented indicate that a preliminary increase in the mean size of the red corpuscles is very frequent in pernicious anemia during liver

therapy. This serves to explain why the correlation between red cell count
and mean corpuscular volume is less marked in the more advanced stages
of anemia when the variations in the size of the corpuscles disturb the reg-
ular pattern of increasing red cell count and decreasing cell size that is so
characteristically seen as treatment becomes effective. The early occur-
rence of the increased macrocytosis, its close correlation with the reticulo-
cyte curve, and the lack of evidence of significant alterations in the number
of microcytes or small poikilocytes, or in the intensity of blood destruction,
strongly suggest that the preliminary increase in mean corpuscular volume
during liver therapy is due, in great part at least, to the flooding of the
circulating blood with immature red corpuscles.

In sprue (1) the writer has observed variations in the volume of the
red corpuscles which were similar in every way to those described in per-
nicious anemia. In these cases there appeared to be the same close paral-
lelism between the augmented macrocytosis and the increase of reticulo-
cytes. In several other (unpublished) cases of non-Addisonian macrocytic
anemia an increase in reticulocytes has likewise been associated with an in-
crease in mean corpuscular volume.

Long before the study of reticulocytes had attained the significance it
now holds, Biffi (5) and Hawes (6) observed that the majority of reticulo-
cytes are larger than non-reticulated cells in the same preparation. Key
(7) confirmed these observations but pointed out that reticulocytes of
normal and even of less than normal size may be encountered. More re-
cently this subject has been carefully studied by Persons (8). He demon-
strated that in normal blood, in pernicious anemia and in various types of
" secondary " anemia, the mean diameter of the reticulocytes is greater
than the mean diameter of the adult red cells in the *same* blood, although
the reticulocytes of " secondary " anemia may be actually smaller than
normal reticulocytes. In normal blood, reticulocytes were on the average
1 micron greater in diameter than adult corpuscles. In pernicious anemia
they were 2.1μ larger, and in microcytic anemias 0.6μ larger.

Persons' observations are of great importance in the interpretation of
variations in the mean volume of the red corpuscles in various types of
anemia. If it is presumed for the purpose of discussion that red corpuscles
are short cylinders, differences in diameter between reticulocytes and adult
corpuscles of the extent noted by Persons are found to correspond approx-
imately to differences of 25 c.μ, 64 c.μ, and 11 c.μ, in the mean volume
of immature and adult red cells of normal individuals, and of the average
cases of pernicious anemia, and microcytic anemia, respectively. It is ob-
vious, therefore, that it requires the delivery of no very great number of
reticulocytes into the circulating blood to produce a significant increase in
mean corpuscular volume. Thus it may be calculated that an increase in
reticulocytes of 6 per cent (that is, a change from 1 to 7 per cent) would,

from 68 c.μ. to 73 c.μ., in a typical case of microcytic anemia, from 90 to 97 c.μ. in normocytic anemia, and from 106 to 116 c.μ. in a case of pernicious anemia.

The observations in pernicious anemia which have been recorded indicate that increased activity of the bone marrow, as evidenced by an increase of reticulocytes, is *actually* significant in influencing the mean corpuscular
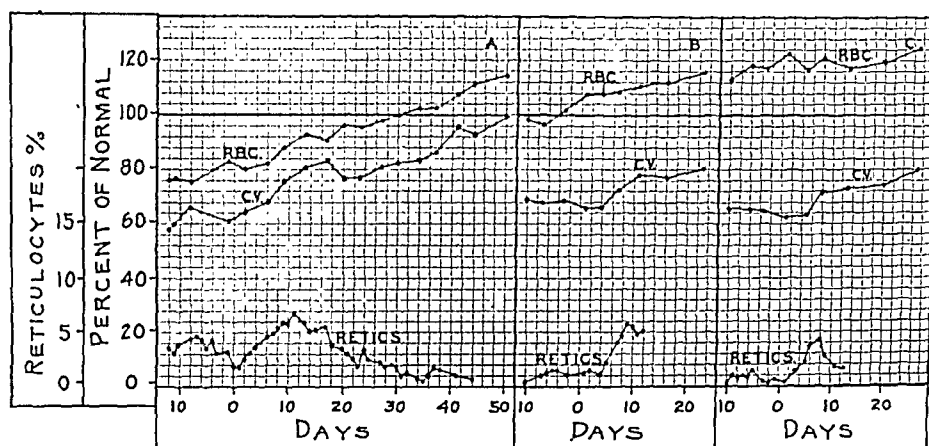
FIG. 4. IDIOPATHIC HYPOCHROMIC ANEMIA. VARIATIONS IN MEAN VOLUME OF RED CORPUSCLES COMPARED WITH RETICULOCYTE COUNT IN THREE CASES.

The abscissa records days preceding and following iron therapy. Legends are in other respects the same as in Figure 1.

volume. There can be little doubt that this is true for other types of anemia as well. In simple microcytic anemia (9) changes in mean corpuscular volume are slight as long as the cause of the anemia persists and the activity of the bone marrow continues to be poor. In hypochromic microcytic anemia, however, the administration of large amounts of iron is associated with a distinct reticulocyte response. In Figure 4 the variations in mean corpuscular volume and reticulocytes in three cases of idiopathic hypochromic anemia are shown. The parallelism between the early and rapid increase in the mean volume of the red corpuscles and the reticulocyte increase in these patients is clear. In a patient with the same type of anemia observed during pregnancy (10) a marked reticulocyte response was associated with a temporary conversion of the microcytic anemia to macrocytic anemia.

It has been pointed out elsewhere (9) that an increase in mean corpuscular volume above normal may occasionally be encountered in cases in which a normocytic type of anemia is usually found; that is, in anemia due to acute blood loss, acute blood destruction, or lack of blood formation. It has been observed, however, that the macrocytosis which may develop in these cases is not as great as occurs in pernicious anemia and that liver therapy is not followed by the spectacular response which occurs in

pernicious anemia and sprue. It has been furthermore noted that in such cases, whenever macrocytosis develops there is evidence of unusually increased blood formation. In view of the evidence concerning the relationship of reticulocytosis to increased mean corpuscular volume, it is very probable that the macrocytosis observed in these cases is due to the appearance of immature red corpuscles in great numbers.

A great increase in the activity of the bone marrow with the delivery of a large number of immature erythocytes into the circulation may, then, lead to the development of macrocytic anemia under circumstances in which normocytic anemia is usually encountered. However, the data available do not indicate that the macrocytosis in pernicious anemia is due entirely to the presence of immature red corpuscles as these are generally understood; namely, nucleated red cells and cells containing reticulum, Howell-Jolly bodies, Cabot ring bodies or refractile (Isaacs) granules. The work of Castle, Townsend and Heath (11) and others (12, 13, 14, 15) has demonstrated the relation of a defect in the formation of a " hematopoietic principle " to the development of macrocytic anemia in pernicious anemia, sprue and certain instances of anemia in pregnancy and " tropical " anemia. It is consequently important to distinguish macrocytic anemia which develops as the result of a fundamental disturbance in the mechanism of blood formation from macrocytosis which simply represents a very active response to the sudden loss or destruction of blood, or is associated with the crowding out of immature red corpuscles from the bone marrow by leukemia or new growths.

SUMMARY

The variations in mean corpuscular volume in cases of pernicious anemia during the response to liver therapy are described.

There is very frequently a temporary increase in macrocytosis following the institution of liver treatment.

This increase in mean corpuscular volume appears to be closely related to the delivery of immature erythrocytes (reticulocytes) into the circulating blood.

The relation of increased activity of the bone marrow to variations in mean corpuscular volume in different types of anemia is considered.

It is suggested that there are two mechanisms by which macrocytic anemia may develop; namely (1) as the result of a defect in the formation of the " hematopoietic principle " of Castle; and (2) by the delivery of large numbers of immature erythrocytes into blood which previously contained red cells of normal size.

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THE DIAGNOSTIC IMPORTANCE OF THE HETEROPHILE ANTIBODY TEST IN LEUKEMIA

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With the demonstration by Paul and Bunnell (1) of an increased titer of heterophile antibodies in the blood serum of patients suffering from infectious mononucleosis, the heterophile antibody test has been introduced into the field of clinical usefulness. Previous to the observations of these authors in 1932, it had been recognized that the blood of most normal individuals contained, in low concentrations, heterophile antibodies, in the form of agglutinins and hemolysins for sheep red blood cells. Following the administration of horse serum these antibodies appeared in increased titer (Davidsohn (2)). Paul and Bunnell (1) reported four cases of infectious mononucleosis which showed heterophile agglutinins to a markedly increased titer. The essential features of their observations have been confirmed by Rosenthal and Wenkebach (3), Boveri (4), Bunnell (5) and Bernstein (6).

It is the purpose of this report to call attention to the very low titer of heterophile antibodies which exists with great constancy in the blood serum of patients with leukemia, to indicate the value of the heterophile antibody test in some conditions differentiated clinically from leukemias with difficulty, and to correlate the findings with previously known data concerning bacterial antibody responses in leukemias.

METHODS

The technical details and symbols by which the results are recorded are identical with those described by Bernstein (6) in a previous paper which may be consulted.

Heterophile antibodies in conditions other than leukemia

In normal individuals, and in patients suffering from a variety of diseases, sheep cell agglutinins may be present in dilutions ranging from 1 to 1 up to 1 to 16. Occasionally there will be no agglutination even in the 1 to 1 dilution. In a group of over 300 such cases, 30 per cent showed no agglutination above 1 to 2 while 70 per cent fell in the range from 1 to 4 up to 1 to 16 (6). For purposes of convenience, the range below 1 to 4

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will henceforth be referred to as Zone 1. The interval from 1 to 4 up to 1 to 16 inclusive will be Zone 2, while the distribution above 1 to 16 will be Zone 3. Thirty per cent of the control group, then, fall in Zone 1; 70 per cent in Zone 2. The great majority of cases of infectious mononucleosis as well as many individuals recently treated with horse serum, appear in Zone 3 (Figure 1).

Normal			Infectious mononucleosis
Zone 1	*Zone 2*		*Zone 3*
←1 : 1 1 : 2	1 : 4 1 : 8 1 : 16		1 : 32 1 : 64 1 : 128 1 : 256 1 : 512→
Leukemia	Serum therapy		

FIG. 1. HETEROPHILE ANTIBODY TITERS UNDER VARIOUS CIRCUMSTANCES

Heterophile antibodies in diseases which may simulate leukemia

Under certain circumstances the following diseases may so closely resemble one of the forms of leukemia that clinical differentiation is difficult: acute infections with hyperleukocytosis; miliary tuberculosis; thrombocytopenic purpura; agranulocytic angina; aplastic anemia; erythroleukemia; infectious mononucleosis; Hodgkin's disease; lymphosarcoma; and carcinoma, tuberculosis or syphilis involving lymph glands.

It has already been noted that in infectious mononucleosis heterophile antibodies usually fall in Zone 3. In two or more instances of the rest of the above mentioned maladies except lymphosarcoma, the antibodies are not necessarily confined to Zone 1, but may fall in Zone 2 as well. Unfortunately only one case of lymphosarcoma has been available for study so that no valid conclusions can be drawn concerning it.

Heterophile antibodies in leukemia

In all the forms of leukemia that were studied, with the exception of one case, heterophile antibodies were limited to Zone 1 (Table I). The single instance in which the agglutinins were encountered in Zone 2 was found, at autopsy, to represent a chronic myeloid leukemia complicated by an osteomyelitis of the jaw and a healed bacterial endocarditis of the aortic valve (Case 13, Table I). In the remaining twenty cases which included acute and chronic forms of lymphoid and monocytic, as well as chronic myeloid leukemia, heterophile antibodies were uniformly low. This rule holds true irrespective of whether or not the patient has received therapy of any sort, including radium or irradiation. For example Case 16 (Table I) when first seen, had a leukocyte count of 400,000. Her heterophile agglutinins were 1 to 2. Five weeks later, after intensive x-ray therapy, her

TABLE I

Heterophile antibody titers in twenty-one cases of leukemia, both treated and untreated

Case	Type of leukemia	Leukocyte count	Heterophile antibody titer *					
			Zone 1			Zone 2		
			1 : 1	1 : 2		1 : 4	1 : 8	1 : 16
1	Acute lymphoid	12,000				−	−	−
2	Acute lymphoid	15,000	+	−		−	−	−
3	Acute lymphoid	106,000	+	±		−	−	−
4	Chronic lymphoid	980,000				−	−	−
5	Chronic lymphoid	150,000	−	−		−	−	−
6	Chronic lymphoid	300,000				−	−	−
7	Chronic lymphoid	100,000	−	−		−	−	−
8	Chronic lymphoid	20,200	−	−		−	−	−
9	Chronic lymphoid	63,000	+	−		−	−	−
10	Chronic lymphoid	8,800	+	±		−	−	−
11	Chronic lymphoid	190,000	−	−		−	−	−
12	Chronic lymphoid (leukosarcoma?)	44,500	+	−		−	−	−
13	Chronic myeloid	25,000				+ +	±	−
14	Chronic myeloid	37,000				−	−	−
15	Chronic myeloid	17,800	+	±		−	−	−
16	Chronic myeloid	400,000	+ +	+		−	−	−
17	Chronic myeloid	102,000	+	±		−	−	−
18	Chronic myeloid	15,850	−	−		−	−	−
19	Chronic myeloid	800,000	−			−	−	−
20	Acute monocytic	8,000	+	±		−	−	−
21	Chronic monocytic	10,800				−	−	−

* Blank spaces indicate that no determinations were carried out at these dilutions.

white blood cell count had fallen to 10,000 but the heterophile agglutinins remained 1 to 2.

Instances in which heterophile antibody determinations proved helpful in clinically confusing conditions

The relevant details of two cases in which doubt existed as to whether or not a leukemia was present, will be presented. In both of these, the conclusions derived in part from heterophile agglutinin determinations were in accord with the diagnosis based on autopsy or biopsy.

Case 22. S. F., a white male, tire builder, aged 36 years, was admitted to the Johns Hopkins Hospital on March 24, 1933 complaining of shortness of breath and weakness. For seven years preceding the onset of the present illness he had worked as an automobile tire builder in which capacity he was constantly exposed to the fumes of benzol.

Early in January, 1933 he developed an acute respiratory infection with a chill, fever, cough and soreness in the chest. From this attack he recovered within a week but from that time on he became weak, suffering from sweats and dyspnoea on exertion.

On admission his temperature was 103° F. He appeared chronically ill. There was a waxen pallor of the skin and the mucous membranes were almost colorless. In the mouth and over the upper trunk were numerous petechiae. There was slight general glandular enlargement. Neither spleen nor liver could be felt.

The patient's course was steadily downhill. It was characterized by irregular fever, various hemorrhagic phenomena and progressive weakness. He died on May 4, 1933.

Examination of the blood at the time of admission showed a depression of all elements with an increase of young myeloid cells. Red blood cells were 3,530,000; hemoglobin was 40 per cent; the leukocytes were 3,800; platelets were 42,000. There was prolongation of the bleeding time, clotting time and the clot retraction. A differential count showed myeloblasts 4 per cent; myelocytes 35 per cent; juvenile neutrophils 19 per cent; segmented neutrophils 5 per cent; basophils 2 per cent; young lymphocytes 25 per cent; adult lymphocytes 10 per cent. Repeated blood cultures were sterile.

The difficulties in diagnosis were obvious. The problem was tersely stated by one observer as follows: "A most confusing case! The story suggests, among other things, benzol poisoning. The blood picture indicates rather an acute myeloid leukemia (aleukemic). The clinical picture might be either of these. It seems to me most likely that this is a case of acute myeloid leukemia (aleukemic) in a man who happens to have been exposed to benzol."

At autopsy there were petechial hemorrhages throughout the organs but no evidence of cellular infiltration. The bone marrow was hyperplastic but full of abnormal young white blood cells. The hyperplasia was considered to have developed because the patient had been removed from benzol for some months before his death, which gave the bone marrow opportunity to attempt to regenerate. The pathological diagnosis was aplastic anemia due to benzol poisoning.

During the patient's stay in the hospital three determinations of heterophile antibodies in his blood serum were carried out. The titer varied between 1 to 8 and 1 to 16, evidence according to previous observations that the disease was not a leukemia.

Case 10.[1] A. M., a white female, housewife, aged 30 years, was admitted to the Union Memorial Hospital on November 4, 1933 complaining of fever and weakness. The illness had begun in March 1932 with pains in the extremities. Examination of the blood in November 1932 showed no anemia but there was revealed a leukocytosis of 21,600 of which 76 per cent were lymphocytes. At that time her general condition was so excellent that it seemed unlikely that she could be suffering from leukemia. She was given Fowler's solution under which therapy there was subjective improvement. However, she developed progressive anemia, became gradually weaker and finally hospitalization was necessary.

On admission to the hospital in November 1933 her temperature was 101° F. She was notably pale and weak. There was no glandular enlargement but the spleen was moderately increased in size. Examination of the blood showed 2,700,000 red blood cells, hemoglobin 50 per cent, and 8,800 white blood cells of

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[1] For the privilege of quoting the data in this case I am indebted to Dr. John L. Dorsey.

which 25 per cent were polymorphonuclear neutrophils, 74 per cent small lymphocytes and 1 per cent monocytes.

The course of her disease was characterized by fever and continued bone pains. Leukocyte counts varied between 8,000 and 22,000 but were usually below 10,000. She was treated with transfusions, liver extract, pentnucleotide and irradiation; all to no avail. An axillary lymph node, removed on November 6, showed an increase of lymphocytes but was not typical of leukemia. Although an aleukemic lymphoid leukemia seemed the most probable diagnosis, splenectomy was carried out on November 14 with the hope that an obscure infection or an atypical Banti's disease might be improved by this measure. The microscopic sections of the spleen showed unmistakable signs of lymphoid leukemia. The patient failed gradually so that in April 1934 she was close to death, with a hemoglobin of 10 per cent.

At the time of splenectomy, sheep cell agglutinins in her blood serum were 1 to 2, a value compatible with leukemia.

The effect of horse serum upon the titer of heterophile antibodies in leukemia

In normal individuals the injection of horse serum will elicit, with rare exceptions, an increase in the concentration of sheep cell agglutinins (2). It was deemed proper, therefore, to determine whether or not a patient with leukemia would react similarly. A patient suffering from chronic lymphoid leukemia (Case 9, Table I), was injected intravenously with two 10 cc. doses of horse serum on successive days. Preliminary skin tests proved him not to be sensitive to the material employed. The heterophile agglutinin titer was measured at frequent intervals (Table II).

TABLE II

Effect of intravenous injection of horse serum upon the heterophile antibody titer of the blood serum of a patient with chronic lymphoid leukemia. (Case 9, Table I)

Date	Amount of serum administered	Days after serum	Heterophile antibody titer		
			1 : 1	1 : 2	1 : 4
1933	*cc.*				
September 15............			+	—	—
September 20............	10		+	—	—
September 21............	10	1			
September 23............		3	—	—	—
September 25............		5	—	—	—
September 26............		6	+	—	—
September 28............		8	—	—	—
October 3..............		13	+	—	—

The concentration of sheep cell agglutinins did not show any significant variation from the original value over a period of time in which, in a normal individual, the heterophile antibody titer would have increased.

This same experiment was repeated with a second individual (Case 12, Table I). This was a patient with the hematological findings of lymphoid

Early in January, 1933 he developed an acute respiratory infection with a chill, fever, cough and soreness in the chest. From this attack he recovered within a week but from that time on he became weak, suffering from sweats and dyspnoea on exertion.

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September 23..........		3	—	—	—
September 25..........		5	—	—	—
September 26..........		6	+	—	—
September 28..........		S	—	—	—
October 3.............		13	+	—	—

The concentration of sheep cell agglutinins did not show any significant variation from the original value over a period of time in which, in a normal individual, the heterophile antibody titer would have increased.

This same experiment was repeated with a second individual (Case 12, Table I). This was a patient with the hematological findings of lymphoid

leukemia who in addition had several large abdominal masses, as well as general glandular enlargement, a picture which was considered to conform rather to leukosarcoma than leukemia. An initial one plus heterophile agglutinin titer of 1 to 1 rose to plus minus at 1 to 4 on the eighteenth day after the injection of horse serum. Such an increase is less both in degree and extent than is encountered in normal individuals treated with horse serum. There was no evidence of serum disease in either of the two patients and it is of passing interest to note that the leukocyte counts, during the period of observation, showed no more than the expected oscillations.

DISCUSSION

Heterophile antibodies in the sera of patients suffering from leukemia are present in low titer. In twenty-one cases of leukemia, a group that embraced lymphoid, myeloid and monocytic types, sheep cell agglutinins were confined to Zone 1 with the exception of one case of chronic myeloid leukemia. In this respect all types of leukemia behave essentially the same. However, in various other diseases which may simulate leukemias, heterophile antibodies were distributed normally in Zones 1 and 2. As an aid in differential diagnosis, this peculiarity of leukemic serum is of definite value. With heterophile agglutinins present in Zone 2 one can conclude with some assurance that the patient whose serum is being tested is *not* suffering from a leukemia. With the agglutinins confined to Zone 1 the unknown serum may or may not indicate a leukemia. The test gives only negative evidence. By its use a leukemia can probably be ruled out but it will not definitely establish the existence of a leukemia. Case 22 is an example wherein even negative evidence is of importance. The presence of sheep cell agglutinins in Zone 2 suggested that the disease process was not leukemia, a conclusion which was subsequently substantiated by pathological examination. In Case 10 one could only say that the agglutinin titer was compatible with leukemia.

That the mechanism whereby antibodies are formed is disturbed in leukemia has been recognized. In 1914 Moreschi (7) obtained little or no agglutinin response to the injection of typhoid vaccine in eight cases of leukemia. For this observation he offered two possible explanations: that the abnormal leukocytes circulating in the blood stream destroyed the antigen; or that the antibody forming ability of the bone marrow was suppressed by the leukemic process. In the same year Rotky (8) injected a strain of vibrios, to which patients with various diseases responded with agglutinins, into two patients with leukemia. Here again no agglutinins were elicited. He suggested a possible relationship between the inadequate antibody response in leukemia and the susceptibility of patients suffering from this disease, to infections. Hickling and Sutliff (9) studied a case of lobar pneumonia occurring in a man with lymphoid leukemia. Although the patient recovered from the acute infection, no agglutinins

for pneumococci were demonstrable in his blood during convalescence. The absence of agglutinins may be encountered, however, in an otherwise normal person with lobar pneumonia.

In some of their manifestations leukemias have the characteristics of invasive tumors.   It is of interest, then, to note that in patients with cancer there was no depression of heterophile antibodies.   Sheep cell agglutinins in several cases of carcinoma originating in the pancreas, as well as in one instance of a hypernephroma with multiple skeletal metastases, were normally distributed in Zones 1 and 2.

SUMMARY

Heterophile agglutinins in the blood sera of twenty-one patients with leukemia were confined to low titers: less than 1 to 4, in twenty instances. In most of the conditions simulating leukemia, heterophile agglutinins were found over a wider distribution of titers up to 1 to 16.   Two clinical histories are given as examples of instances in which this differential point was of assistance in arriving at a diagnosis.

Intravenous administration of horse serum which, in a normal person, elicits an increase in the concentration of heterophile antibodies, failed, in one case of lymphoid leukemia, to raise the heterophile antibody titer.   In a second case of probable leukosarcoma, horse serum brought about a minimal increase of sheep cell agglutinins.   Neither of these patients developed serum disease.

The restriction of heterophile antibody concentrations in leukemia to low titers is in accord with previously known immunological characteristics of individuals with this disease.

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PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., APRIL 30, 1934

Hyperparathyroidism Due to a Diffuse Hyperplasia of All Parathyroid Glands Rather than to a Parathyroid Adenoma of One Gland—Clinical Studies on Two Such Cases. By FULLER ALBRIGHT, EDWARD D. CHURCHILL, and (by invitation) BENJAMIN CASTLEMAN, Boston, Mass.

After an experience with fourteen cases of hyperparathyroidism proven by operation, in which there was every reason to believe the hyperparathyroidism was due to an adenoma of just one of the glands, we encountered patients number 15 and 17 in our series in whom the situation was entirely different. Both of these individuals had typical hyperparathyroidism as judged by metabolic criteria. At operation all the parathyroid glands were found to be enormous, three being encountered in the first patient and four in the second. Removal of two glands in the first patient was without effect on the degree of hyperparathyroidism. Removal of three glands and part of the fourth produced the required effect in the second patient. It is apparent that the condition was a diffuse hyperplasia rather than an adenoma. The histology of the material removed was similar in both patients and differed markedly from that seen in our first fourteen cases. The analogy with Graves' disease is suggested. Certain difficulties in the treatment of such patients are presented. We believe this condition is a separate disease entity due to the presence of a parathyreotropic substance in abnormal amounts.

Observations on Adrenalectomized, Depancreatized Animals. By C. N. H. LONG and (by invitation F. D. W. LUKENS, Philadelphia, Pa.

In five cats we have removed, in stages, all the pancreas and both adrenal glands. Liberal dosage of a commercial cortical extract was immediately instituted but at no time was any insulin given. The periods of survival were 12, 11, 8 and 6 days, while one animal was sacrificed in good health on the 7th day for determination of liver glycogen (2.5 grams per cent).

None of the animals presented the usual clinical picture of those depancreatized with the adrenals present. They remained active and ate fairly well up to the day of death. They have all died suddenly in convulsions.

The most striking features have been: (*a*) the fasting blood sugars were, in the majority of observations, not above normal limits and showed a remarkable tendency after the first few days to fall to such low levels that convulsions, relieved by glucose, have occurred; (*b*) glycosuria was slight or absent depending upon the amount and type of diet; (*c*) metabolic balance sheets have been constructed and show that while considerable amounts of the glucose of the diet were utilized yet the tolerance was not normal.

These animals resembled those suffering from adrenal insufficiency in spite of the fact that supposedly adequate doses of cortical extract were administered.

A Study of the Acid-Base Equilibrium in a Patient with Acute Gout. By JOHN H. TALBOTT (by invitation) and ARLIE V. BOCK, Boston, Mass.

A 21-year old male patient suffering from recurrent attacks of gout has been studied on a metabolic regime for more than five months. Twelve complete

cycles, comprising the attack and convalescence, have been investigated in detail. All of them have shown a similar phenomenon in respect to the acid-base changes of the blood and urine. One complete cycle of thirteen days will be reported at this time. This period started three days before the peak of the symptoms of one attack, continued through this attack and its recovery and extended through the prodromal period of the following attack.

On the metabolic regime the fluid and food intake was maintained constant. A low purine diet was given. Every fifth day a complete supply of food was purchased for the following days and a 50 per cent aliquot portion of a whole day's diet was analyzed. These analyses included Na, K, Ca, total inorganic base, P, Cl, and total N. Twenty-four hour urines were collected and analyzed for Na, K, Ca, total inorganic base, NH_3, P, Cl, urate, titratable acid and total N. The stools were collected in periods corresponding to phases of the attack and were analyzed for the same constituents as the diet. Daily venous blood samples were collected and equilibrated at 37.5° C. at a known pH. A portion of the whole blood was analyzed for uric acid and CO_2. The remainder was centrifuged and the separated plasma was analyzed for Na, K, Ca, total inorganic base, CO_2, P, Cl, urate, non-protein nitrogen and total N. The cells were analyzed for Na, K, Cl and H_2O.

One of the significant findings in this study was a diuresis preceding each attack, beginning at least twenty-four hours before any symptoms of acute gout. This diuresis continued after the onset of symptoms and reached a maximum on the third day. On this day, colchicine was administered to alleviate the pain. As the symptoms subsided the urinary output diminished and reached a minimum three days later. In convalescence, the urinary output gradually increased and reached a second maximum with the next attack, thus completing a cycle. The changes in the urinary volume were associated with an increased concentration of Na and Cl not noted in connection with any of the other electrolytes. On a daily intake of approximately 75 m.Eq. of Na and Cl the excretion of these substances was over 125 m.Eq. on the day preceding symptoms. In the recovery phase there was a retention of the above to compensate for the dissipation. Other constituents of the urine which showed changes of excretion approximately proportional to the change in urine volume were K, Ca, P and uric acid. The excretion of NH_3 and titratable acid showed no direct relation to the attacks. Approximate nitrogen balance was maintained through the cycle.

Regarding the constituents of the plasma, there was a gradual elevation of K from 3 m.Eq. to a peak of 6 m.Eq. at the height of the attack. After the peak the CO_2 increased 3 m.Eq. and the Cl decreased 6 m.Eq. Cell volume and plasma protein concentration showed little change.

In summary, changes of considerable magnitude in the acid-base balance of the body have been observed in a patient suffering from acute gout, the interpretation of which is subject to further study.

Skin Temperature Changes Following Food Digestion. By GEORGE BOOTH and J. M. STRANG (by invitation), and FRANK A. EVANS, Pittsburgh, Pa.

Variations of skin temperature following a meal of meat, designed to attain satiety, were made on twenty normal and thirteen obese patients. The Tycos dermatherm was used and each observation represents the average of two to four determinations at four selected points.

Average of findings were:

	Meat eaten	Duration of eating	Skin temperature			
			Elevation began after	Elevation at time of satiety	Maximum elevation attained after	Maximum elevation
	grams	*minutes*	*minutes*	° C.	*minutes*	° C.
Normal..	505	24	6	0.9	54	1.9
Obese...	495	25	25	0.1	45	0.6

Only four of the twenty normal and five of the thirteen obese failed to show the characteristic pattern of curve.

Satiety, marked by a distinct sensation of warmth in most cases and by the appearance of gross perspiration in some, is probably related to the response of skin temperature to food. The depression and delay of this reaction, observed in the obese would permit of the ingestion of much larger quantities of food than would occur in persons of normal nutritional state.

A Peculiarity in Carbohydrate Metabolism of Cancer. By FREDERICK H. SCHARLES, and DWIGHT BAKER (by invitation), and WILLIAM T. SALTER, Boston, Mass.

High lactic acid production is known to be a common characteristic of intact malignant tissue. Nevertheless, no extract of malignant tissue has hitherto been found capable of producing lactic acid from glucose or glycogen. When a saline extract of mouse sarcoma No. 180 is incubated with a phosphate-buffered glycogen solution, the glycogen is hydrolyzed but no lactic acid is produced. Fresh muscle extract, on the other hand, incubated with a similar substrate, forms appreciable amounts of lactic acid. If, however, hexose diphosphate or hexose monophosphate (prepared from yeast) are used as substrate, tumor extract splits them with the production of lactic acid, just as does muscle extract.

This finding suggests that there is a deficiency in the enzyme system of extracted malignant tissue which has not hitherto been described. The reasons for postulating this deficiency are as follows: First, because it has been previously shown that a glycogen-splitting enzyme is present in sarcoma extract. Secondly, because Lohman's "coenzyme" (essential to lactic acid formation) is present in tumor as well as in muscle. Consequently, by elimination, the defect may lie in the inability of sarcoma extract to synthesize a precursor of lactic acid, possibly a hexose phosphate.

The State of Calcium in the Blood in Rickets.[1] By EDWARD L. COMPERE (by invitation), FRANKLIN C. MCLEAN, and (by invitation) A. BAIRD HASTINGS, Chicago, Ill.

Fifty-two observations were made upon the blood serum of twenty-one children diagnosed as with rickets. The children varied in age from five months to two and one-half years, and the diagnoses varied from very mild or doubtful to very active rickets. The study was begun before any treatment was instituted and extended into a period of about five weeks' treatment with cod liver oil or irradiated yeast; a control group remained untreated throughout.

Total protein, inorganic phosphate, total calcium and calcium ion were determined simultaneously on each sample of blood (taken fasting), the first three by the conventional chemical methods. Calcium ion concentrations were observed directly by the frog-heart method (1), and calculated from the mass-law equation (2) previously described by two of the authors.

Total calcium, and calcium ion concentrations, both observed and calculated, were found to be in or very near the normal range throughout, and no correlation was found between the calcium findings and the severity of the rachitic process, as judged either by the inorganic phosphate concentration or by all clinical evidence available.

In the absence of the finding of any deviations from the normal or of any correlation with the severity of the rachitic process, it is concluded that the pathological state in rickets is apparently not to be explained upon the basis of any change in the state of calcium in the blood discoverable by this method.

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Observations on the Arginase Activity of Human Blood, with Particular Reference to Blood from Patients Suffering from Malignant Disease or Pernicious Anaemia. By JAMES A. DAUPHINÉE (introduced by Duncan Graham), Toronto, Canada.

A series of quantitative observations on the arginase content of human bloods has been made in normal individuals and in patients suffering from various types of disease. The enzyme has been found to be present in the red cells of all human bloods examined and to be practically absent from all samples of serum. It has been found that the red cells must be completely broken down with a haemolytic agent, such as saponin, before their full arginase activity becomes apparent. No evidence has been found to support the contention that patients suffering from malignant disease show any increased arginase activity. As a general rule, patients who are cachectic, whether from malignant disease or other cause, tend to show lowered arginase values in the blood.

On the other hand, many patients who have severe or moderately severe pernicious anaemia show a marked increase in the arginase activity of the red cells. This may reach as high as ten times the normal value. With adequate liver therapy, this arginase activity of the red cells decreases rapidly at first and then more slowly approaches normal values. The high values found in the red cells of patients suffering from pernicious anaemia are not approached by those found in any other type of disease, including severe secondary anaemias and haemolytic icterus, so far examined.

No evidence has been obtained, therefore, that the arginase content of the blood is of any significance, diagnostic or otherwise, in patients thought to be suffering from malignant disease. The results obtained, however, with the bloods of patients suffering from pernicious anaemia are highly interesting. The reasons for the high arginase activity in the red cells of these patients is not at once apparent. Although an increased arginase content has been observed in the blood of most patients who have this disease with moderate to severe anaemia, yet there does not seem to be any direct relationship between the magnitude of this increase and the severity of the anaemia. In view of the fact that arginase has been shown by Edlbacher and others to be present in

rapidly growing tumors, embryonal cells and young granulation tissue, we have considered the possibility of a relationship between the number of immature red cells in the circulation and the arginase content. Any such relationship, however, is discounted by the lack of parallelism between the arginase activity and the reticulocyte count, both in patients who have pernicious anaemia and in those having a reticulocyte response from other causes. It is probably more likely that the increase in arginase content is due to some abnormality other than immaturity of the red cells, which would seem to be peculiar to pernicious anaemia and which is abolished by treatment with liver. We are making this the subject of further study.

Studies on the Mechanism of Hyperthyroidism.[1]   By DONALD McEACHERN and E. COWLES ANDRUS, Baltimore, Md.

Using the Warburg-Barcroft technique, measurements were made of the respiration of representative tissues from normal and thyroxine-injected rats. At intervals of 22 hours to 8 days following a single injection of thyroxine (10 mgm. per kilo.) the following changes of tissue respiration were found in 11 experiments: Mean Q_{O_2}.[2] Kidney: Normal $= 21.50$; Hyperthyroid $= 27.62$ ($+28$ per cent). Liver: N. $= 10.39$; H $= 12.88$ ($+24$ per cent). Diaphragm: N $= 4.93$; H $= 5.12$ ($+4$ per cent).

In 12 experiments following multiple injections of thyroxine (10 to 15 mgm. per kilo. from twice in 5 days to 8 times in 18 days) much greater increases of oxygen consumption were obtained, the mean Q_{O_2} being Kidney: N $= 23.72$; H $= 34.65$ ($+46$ per cent). Liver: N $= 9.59$; H $= 16.62$ ($+73$ per cent). Diaphragm: N $= 4.71$; H $= 8.32$ ($+76$ per cent). In all subsequent experiments hyperthyroidism was induced by multiple injections of thyroxine. In 13 experiments a comparison was made between the total metabolism of intact animals and the respiration and anaerobic glycolysis of their isolated tissues. A fair parallel was found in the oxygen consumption. The mean basal metabolic rate of hyperthyroid animals was $+84$ per cent and the increased Q_{O_2} of their tissues was: kidney $+61$ per cent, liver $+46$ per cent, diaphragm $+53$ per cent. The anaerobic glycolysis was increased 90 per cent in kidney but in other tissues showed no significant change. In 15 experiments, methylene blue (0.05 per cent) caused similar changes in the respiration of normal and hyperthyroid tissues but the glycolysis of normal kidney and liver was accelerated three times more than in hyperthyroid tissues.

The respiration of hyperthyroid cells (in phosphate buffer) was more sensitive to cyanide than that of normal cells and with a concentration of $M/2800$ NaCN the former was inhibited to a well sustained normal level. KI in concentrations of $1:500,000$ and $1:1,000,000$ had no significant effect on either normal or hyperthyroid tissues. Fluoride (0.002 to 0.0002 M) and iodoacetic acid (10^{-5} to 10^{-6}) reduced the respiration of hyperthyroid cells to well sustained normal levels. Daily injections of fluoride and iodoacetic acid into hyperthyroid animals failed to prevent their decline and death. Muscle enzyme extract from normal and hyperthyroid animals when added to normal tissues caused approximately equal increases of respiration apparently due to the presence of metabolites. Hyperthyroid tissues were able to utilize sodium pyruvate, sodium d-lactate and sodium succinate (all 0.018 M) equally as well as normal

tissues and responded by similar percentage increases 'of respiration of from 50 per cent to 150 per cent. Succinate caused the greatest increases. Dihydroxyacetone (0.03 M) had no constant effect on the respiration of normal or hyperthyroid cells.

It was concluded that:

1. Representative tissues from hyperthyroid animals preserve an increased oxygen consumption after isolation from the animal body.

2. The increase in tissue respiration bears a fair parallel to the increase of total oxygen consumption by the intact hyperthyroid animal.

3. The increased moiety of respiration in hyperthyroid tissues is not carried out by any new or abnormal respiratory mechanism in the cell.

4. No increase in the inherent ability of the hyperthyroid organism to produce lactic acid can be demonstrated and an increased rate of glycolysis is not the fundamental cause of the increased oxygen consumption.

5. Cyanide, fluoride and moniodo-acetic acid reduce the excess respiration but do not affect the fundamental mechanism which makes an increased supply of oxygen necessary to the organism.

6. Hyperthyroid tissues are able to burn various substrates (lactate, pyruvate, succinate) as well as do normal tissues.

The Calorigenic Action of Various Thyroid Derivatives. By W. O. THOMPSON and (by invitation) S. B. NADLER, S. G. TAYLOR III, and P. K. THOMPSON, Chicago, Ill.

We have found that, after peptic digestion of desiccated thyroid, about eighty per cent of its activity is possessed by the acid-insoluble portion, which contains less than half of the total iodine. The remaining activity is possessed by the acid-soluble portion, which contains the rest of the iodine. The curves denoting the change of the basal metabolism following single large doses of these two portions seem to be different. The activity of desiccated thyroid may not be proportional to its total iodine content, but only to a fraction of the iodine which possesses nearly all of the calorigenic activity. An iodine compound has been prepared from desiccated thyroid which, when given by mouth, possesses greater calorigenic activity per milligram of iodine than desiccated thyroid given orally and in some instances greater than thyroxine given intravenously.

Heating desiccated thyroid with alkali greatly reduces its activity, whereas this treatment does not affect the activity of thyroxine. Possible explanations are: 1. The active principle of the thyroid may be present in some form or forms other than thyroxine. 2. Thyroxine, when combined with other amino-acids in a protein molecule may be more susceptible to destruction than when present as the simple amino-acid.

The Standard Physiology of Extreme Old Age. By HOWARD F. ROOT and (by invitation) FRANCIS G. BENEDICT, Boston, Mass.

The pathology and degeneration of old age are always before us. Observations upon a man 91 years of age disclose some of the physiologic phenomena of "ideal" old age. Mr. L. showed an extraordinary preservation of physical and mental power including libido normal for a man in youth. The basal metabolism showed an average oxygen consumption of 153 cc. per hour and respiratory quotient of 0.77. The heat production of 18.3 calories per kilogram, or 629 calories per square meter is the lowest for all men examined at the Carnegie Nutrition Laboratory. It was 31 per cent lower than the average for 30 men of the same height and weight but younger in age. The depression of

metabolism in extreme old age may not be properly expressed in the Aub-DuBois or Dreyer standards.

The low skin temperature of the feet 27.2° C. in spite of good pulsation in the dorsalis pedis arteries suggests the factor of slow blood flow.

The insensible perspiration, blood chemistry, urine chemistry, hematology and roentgenograms demonstrating absence of calcification in the arteries are reported.

Changes in the Minute Volume Output of the Heart and Urea Clearance in Relation to Age. By WILLIAM H. LEWIS, JR., and ALF S. ALVING (introduced by Oswald T. Avery), New York, N. Y.

Twenty normal active men in each decade of age from the 5th to the 10th have been studied. The observations to be reported included measurements of the minute volume output of the heart, the function of the kidneys illustrated by urea clearance, curves of the consumption of oxygen, vital capacity of the lungs, action of the heart manifested electrocardiographically, and data concerning the size and shape of the heart and the thoracic aorta as seen in x-ray photographs.

The curves show that the behavior of the human organism alters significantly after the 65th year. The basal consumption of oxygen, the vital capacity, and the renal function decrease; their decline presents a parabola like curve. The minute volume output of the heart under basal conditions and corrected to body size remains at the level for adults between the ages of 20 and 40 years.

The study considers aspects of physiological evolution by simultaneous analysis of the functions mentioned over a given period of life.

Quantitative Tissue Reactions in Normal and Immunized Rabbits Following the Intravenous Injection of Non-Pathogenic and Pathogenic Bacteria. By ROBERT N. NYE, Boston, Mass.

The rate of disappearance of intravenously injected *B. subtilis* from the blood stream, the localization of the bacteria in the lungs, liver, spleen and bone marrow and the changes in the distribution of the polymorphonuclear leukocytes in the blood, lungs, liver, spleen and bone marrow, all in normal rabbits, serve as a basis for comparison.

The rapid disappearance of polymorphonuclear leukocytes from the blood stream appears to be directly proportional to the rate of removal of bacteria from the circulation in all experiments: intravascular phagocytosis, however, is extremely rare. The removal of the spleen does not affect the rapid clearing of the blood stream of bacteria, nor does superimposed blocking of the hepatic circulation. Staphylococci are removed about as rapidly as hay bacilli, but dead and live pneumococci either persist for longer periods or actually increase. Both dead and living pneumococci, however, disappear in immunized animals with a rapidity nearly comparable to that of other bacteria, although immunization has no affect upon the removal rate with *B. subtilis*.

The localization of polymorphonuclear leukocytes in any organ appears to be directly proportional to the number of bacteria found in that organ. Whereas phagocytosis by these leukocytes, primarily in the lungs and liver, is relatively common following the injection of hay bacilli and staphylococci, it is seen only rarely with pneumococci. The latter are phagocytized chiefly by cells of the reticulo-endothelial system in the liver and spleen; but this process is accompanied by the localization of polymorphonuclear leukocytes.

The Influence of Lipoids in Tissue Immune Reactions. By FRANKLIN M. HANGER. New York, N. Y.

Immune responses are traditionally measured by serological changes. However, it is well recognized that some of these responses are demonstrable only by an altered tissue reactivity. The influence of lipoids on the serological changes of immunized animals has been frequently studied, but their effect on tissue reactivity has not been adequately investigated.

A group of rabbits has been vaccinated intradermally with living and heat-killed strains of hemolytic streptococcus. Another group of animals received identical doses of vaccine to which various lipoids were added. The chief effects noted were:

1. The sites of intradermal vaccination healed more promptly with lipoids present.

2. Allergic response as expressed by skin tests with bacterial proteins seemed diminished in the lipoid treated animals.

3. Animals injected with streptococcus alone showed no immunity when subsequently infected with a virulent strain of *B. lepisepticum.* However, many animals treated with the streptococcus lipoid mixture showed immunity, though no demonstrable protective antibodies developed in their sera.

4. Purified lipoids failed to produce the effects noted above.

5. If rancid lipoids were injected with the vaccine a reversed influence was observed and the resistance of the animal was markedly diminished.

6. Intravenous injections of lipoids had no demonstrable effect on vaccination.

Cutaneous Reactions in Pneumonia to the Somatic ("C") Polysaccharide of Pneumococcus. By THOMAS FRANCIS, JR. and (by invitation) T. J. ABERNETHY, New York, N. Y.

The capsular polysaccharide of pneumococcus is type-specific; its intradermal injection elicits an immediate wheal and erythema reaction in the skin of patients with pneumonia at the onset of recovery. The reaction is type-specific and occurs only in the presence of homologous type-specific antibodies.

The somatic carbohydrate, or "C" fraction, is not type-specific. It is derived from pneumococci of all types or even from degraded unencapsulated forms. During the acute phase of pneumonia, precipitins to the somatic carbohydrate are demonstrable in the serum of pneumonia patients, but disappear with recovery. Similarly, skin reactions to the somatic carbohydrate are elicited during the febrile stage of pneumonia and become negative in convalescence.

The cutaneous response first appears as an immediate wheal and erythema, which is followed by the delayed reaction, an edematous erythema. The center of the delayed reaction is frequently dark, reddish brown, sometimes hemorrhagic; about this there may be a pale white halo; and beyond this a bright red erythema. The delayed reaction begins to appear in 2 to 3 hours, is well marked in 6 to 10 hours, persists for about 18 hours, and then fades leaving a residual brown stain. The reaction may vary from 1 to 5 cm. in diameter.

In general, the reaction is related to the presence of precipitins in the serum, and is apparently a true antigen-antibody reaction. It may have a prognostic significance, in that cases which terminate fatally fail to give a positive skin test. The reaction is not specific, since it may also be elicited in certain other acute infections, such as rheumatic fever, but not in all febrile states. Normal human subjects usually give no response.

Reappearance of Clinical Signs and Symptoms of Rheumatic Fever Following Non-specific Protein Shock. By T. DUCKETT JONES and (by invitation) EDWARD F. BLAND, Boston, Mass.

Upper respiratory infections frequently precede the onset of clinical recurrences of rheumatic fever by from several days to three or four weeks. However, seemingly unrelated episodes such as tonsillectomy, abdominal operations, accidents and unexplained fever have also been noted. Since protein shock by means of intravenous administration of typhoid-paratyphoid vaccine has been widely used as a therapeutic measure in many diseases, it was thought justifiable to use this means to reproduce the fever (commonly associated with respiratory infection, but occasionally unexplained) which is often observed before the onset of clinical rheumatic fever.

Twelve observations were made upon ten hospitalized patients convalescing from recent rheumatic fever, in whom the clinical symptoms and signs of the disease had subsided, but in whom in most instances there remained laboratory evidence of low grade persistent infection. Non-specific protein shock was induced by the intravenous administration of 0.1 cc. of a stock typhoid-paratyphoid vaccine with resulting chill and rectal temperature of approximately 103° F. In six instances (50 per cent) a recurrence of clinically active rheumatic fever appeared either immediately or after a latent period of one to three weeks. Artificial fever, induced by radiant heat in three of the same group, was not followed by similar recurrences. It is evident that the signs and symptoms of clinical rheumatic fever (recurrent) may follow the use of non-specific protein shock.

Scarlet Fever and Rheumatic Fever. By JOHN R. PAUL, New Haven, Conn.

Recent emphasis upon the rôle of *Streptococcus hemolyticus* infections as a contributing or "activating" cause of rheumatic fever, has made it worth while to analyze certain late complications of streptococcus infections and to compare them to rheumatic fever. Scarlet fever is one of the streptococcus infections often followed by complications, such as arthritis and heart disease which resemble the arthritis and carditis of rheumatic fever. How close this resemblance is has for years been an unsettled question.

Three points of similarity are shown:

(*a*) The clinical course of scarlatinal nonsuppurative sequelae resembles that of rheumatic fever following an upper respiratory infection, and the interval at which arthritis, carditis and nephritis arise following scarlet fever simulates the interval at which rheumatic fever occurs after tonsillitis.

(*b*) From the standpoint of familial epidemiology it is shown that a high incidence of rheumatic fever exists in the families of patients who sustain arthritis and carditis after scarlet fever.

(*c*) A fatal case of post-scarlatinal myocarditis is reported in which Aschoff bodies were found, together with other histological lesions identical with those of rheumatic heart disease.

The significance of this resemblance which these scarlatinal sequelae bear to rheumatic fever is discussed in the light of the pathogenesis of the latter disease.

The Significance of the Relief of Pain Immediately after Complete Removal of the Normal Thyroid Gland in Patients with Angina Pectoris and Congestive Heart Failure. By H. L. BLUMGART and (by invitation) A. A. WEINSTEIN, D. DAVIS and J. E. F. RISEMAN, Boston, Mass.

In treating over sixty patients with angina pectoris and congestive heart failure by removing the entire normal thyroid gland, we have observed that the most striking clinical improvement occurs when the hypothyroid state develops, as gaged by the lowering in the basal metabolic rate. Immediately after operation it has been noted, however, that, at a time before the basal metabolic rate has become significantly lowered or the velocity of blood flow has become slowed, patients very frequently lose localized areas of thoracic tenderness and of constant precordial pain and frequently no longer suffer from attacks of angina pectoris. Extensive studies of this phenomenon have been made for over a year both in patients after bilateral complete thyroidectomy and in patients who have had only one lobe of the thyroid removed at the time of the first operation. These studies will be described.

The observations point definitely to the following conclusions: (1) the immediate relief of pain after total thyroidectomy is due to the interruption of nerve impulses from the heart at the time of operation; (2) relief of pain by this mechanism is only temporary; (3) permanent relief is related to the lessened work of the heart attendant on the development of the hypothyroid state. These findings indicate that after total ablation of the thyroid, complete bed rest should be enforced, despite the early subjective relief experienced by the patient, until the basal metabolic rate shows significant lowering.

The Effect of Theophyllin Ethylendiamin on Experimentally Induced Cardiac Infarction in the Dog. By W. M. FOWLER and H. M. HUREVITZ (by invitation) and FRED M. SMITH, Iowa City, Iowa.

In perfusion experiments on the isolated heart of the rabbit theophyllin ethylendiamin produced a far greater and more consistent increase in the rate of coronary flow than did any of the other drugs tested. This drug was put to a more decisive test by observing its effect on the infarct produced by the ligation of a coronary artery in the dog. Observations were made during the initial stage of the infarction and after an interval of three weeks. In the first series of experiments a coronary vessel was ligated and, after the appearance of the distal area of cyanosis, the drug was administered intravenously. This was followed by a decided reduction in the extent and degree of the cyanosis and, in certain instances, resulted in almost complete restoration of the original color. The drug was then given at daily intervals to a series of animals after the ligation of a coronary artery. These animals were sacrificed after an interval of three weeks, which, in view of the findings, allowed ample time for the maximum restoration of the circulation to the area of infarction. This area was greatly diminished in these animals as compared to that in the control series which did not receive medication and in which a corresponding vessel was ligated at the same level. These results indicate that theophyllin ethylendiamin promotes the development of an extensive collateral circulation.

Cardiac Pain. The Presence of "Pain" Fibers in the Nerve Plexus Surrounding the Coronary Arteries. By L. N. KATZ and (by invitation) W. MAYNE and W. WEINSTEIN, Chicago, Ill.

Sutton and Lueth observed symptoms resembling an "anginal attack" in unanesthetized dogs when a large coronary artery was occluded; an observation

which has been confirmed by Piercy, Priest and Van Allen, and by J. C. White. Sutton and Lueth concluded that the response was precipitated by myocardial ischemia.

Some recent results led us to conclude that this response was due to direct stimulation of the " pain " fibers in the nerve plexus which Woollard found encircles the coronary vessels.   Our evidence for this is:

1. Occlusion of a carefully isolated coronary artery gave no "anginal" response.   However, a definite response was obtained when the compression was applied to the undissected coronary vessels above and below this point.

2. The destruction of the nerve plexus with phenol-alcohol changed a positive to a negative response without affecting the positive response produced by compression of the vessel above the phenolized area.

3. Complete preliminary occlusion of a carefully isolated coronary artery did not prevent a positive response to compression of the vessel above and below this point.

4. Pericardial tamponade, following bleeding from a ruptured coronary artery, caused syncope but no "anginal" response.

The response from the heart was similar to that obtained on compressing a superficial somatic sensory nerve, save for an inability to locate the site of irritation.

The Contour of the Chest as a Factor in the Explanation of the Difference Between the Dog and Human Electrocardiogram.   By M. Prinzmetal (by invitation), W. B. Kountz and D. P. Barr, St. Louis, Mo.

Recent studies by us on revived perfused human hearts have verified the results obtained by Barker, McCloud and Alexander on the electrocardiogram in man.   The explanation of the difference obtained by Barker and Lewis was thus sought.

When the chest plate was removed in the cadaver it was noted that there was no displacement of the human heart, whereas when the dog's chest was opened the heart was displaced posteriorly.   Experiments were designed to study the dog's heart in the anterior mediastinum, and it was found that the electrocardiographic tracings resembled more closely those obtained by Barker in man than those obtained by Lewis in the dog.

The following experiment was performed to study the problem further. The human heart was removed from a cadaver.   Dog's heart-lung preparations were made and the beating dog's heart was inserted into the human pericardial cavity.   The leads were taken from the cadaver's extremities.   Extrasystoles and bundle-branch-blocks were identical to those found in the exposed human hearts.

Since the dog's heart in the human chest and in its normal position in the dog's chest is electrocardiographically similar to the human heart, anatomical differences between the hearts of the two species cannot be used as an explanation for the electrocardiographic differences.   The explanation, therefore, must lie in the difference in contour of the chest of the two species.

Observations on Arterial Function.   By Roy H. Turner and (by invitation) William A. Sodeman, New Orleans, La.

In a study of pulse-wave velocity in which 100 determinations were made in twenty-two normal adults and 82 determinations in thirty-one individuals with hypertension or arteriosclerosis or both, certain observations were made which are not in accord with widely accepted concepts of arterial function:

1. Pulse-wave velocity in normals as a group and repeated determinations in the same group of individuals varied widely, including high values such as: 12.2, 12.0, 11.6, 11.3, 10.8, 10.7 m. per sec.

2. Pulse-wave velocity in some patients with hypertension or arteriosclerosis or both were as low as: 4.1, 4.2, 4.3, 5.7, 4.8, 4.9, 4.4, 5.1, 6.1 m. per sec.

3. Fall in blood pressure was frequently associated with a rise in pulse-wave velocity.

4. In our normal group pulse-wave velocity tended to fall as pulse rate rose.

Explanation of high pulse-wave velocity in normal arteries with normal blood pressure and of low pulse-wave velocity in thickened arteries, with or without high blood pressure, depends upon changes of tone of smooth muscle in the arterial walls or changes in diameter of arterial lumen or both.

These findings indicate that until conditions have been defined which insure maximum elasticity of which the artery is structurally capable, determinations of pulse-wave velocity will be of little aid in detecting the presence of arteriosclerosis and measuring its degree.

The Cerebral Circulation. XXXII. The Effect of Sympathetic Nerve Stimulation on the Pial Vessels in the Isolated Head. By J. L. Pool (by invitation) and H. S. Forbes, Boston, Mass.

It has been shown that stimulation of the cervical sympathetic nerve is usually followed by constriction of the ipsolateral arteries of the pia mater, and presumably of the ipsolateral cerebral arteries in addition. Similar results have been attained in experiments on a partially isolated head mechanically perfused by the animal's own blood. In all this work, however, the possibility of an hormonal effect existed, indicating the necessity of an experiment in which a closer approach to physiological conditions prevailed, and which, at the same time, would eliminate all effects other than the direct action of sympathetic nerve stimulation on the intracranial vessels.

Therefore, microscopic measurements of the pial vessels were made in a head completely isolated from its body, circulation being maintained by direct vascular anastomosis with a living intact animal serving as donor.

Sympathetic stimulation in five such preparations resulted in an average constriction of the ipsolateral pial arteries amounting to 9.6 per cent, 19 times out of 21 trials. No change occurred in the remaining 2 trials. No effect on the contralateral vessels was noted. A concomitant decrease in the cerebrospinal fluid pressure of the isolated head amounting to 14 per cent also occurred.

The Etiology of "Alcoholic" Polyneuritis. By Maurice B. Strauss (introduced by Henry Jackson, Jr.), Boston, Mass.

The clinical and pathologic similarity of alcoholic polyneuritis and beri-beri has recently been pointed out. Minot, Strauss, and Cobb have shown that patients with alcoholic polyneuritis usually have had a poor diet and have eaten relatively little food over long periods of time. Frequently gastro-intestinal disturbances occur, possibly due to the effects of excessive alcohol, which may interfere with the absorption of accessory food factors. Fifty per cent of our 43 patients had gastric anacidity, and 25 per cent had pellagra in addition to polyneuritis, which favors the view that dietary deficiency plays a rôle in the latter condition. Spies and De Wolf have shown that "alcoholic" pellagra may be cured during the continuous administration of alcohol by the use of a diet rich in vitamin B_2. This suggested that "alcoholic" polyneuritis might be relieved during the continuous administration of alcohol by a diet rich in

vitamin B₁. Accordingly, seven patients suffering from alcoholic polyneuritis, in one instance complicated by an alcoholic psychosis, have been given a diet rich in the vitamin B complex together with intramuscular injections of vitamin B concentrates. In each instance the patient was furnished with his usual daily allowance of alcoholic beverage, this varying from one pint to one quart of 100-proof spirit. Without exception the patients showed steady improvement under this regime. Pain disappeared. Motor power and sensation returned. Absent reflexes reappeared in one case during the period of observation. The patient with least disturbance was well within two weeks, whereas the most severely paralyzed individual required four months of treatment. These observations constitute definite evidence that pure alcohol does not have a specific neurotoxic effect on the peripheral nervous system. Although the possibility remains that some impurity in the beverage alcohol consumed by these patients before the onset of polyneuritis was responsible for the nerve lesions, all the evidence thus far presented suggests that alcoholic polyneuritis is a dietary deficiency disease similar to beri-beri.

The Abortion of Migraine Headaches by Means of Ergotamine Tartrate. By WILLIAM G. LENNOX, Boston, Mass.

Forty-five patients suffering from migraine have been given ergotamine tartrate (Gynergen) during a headache. All of these patients were chronic cases with frequent headaches which were not relieved by the drugs commonly used. Injection of the ergotamine tartrate was followed by abrupt termination of the headache in question in 40 of the 45 patients, i.e., 90 per cent. In all but a few cases the medicine was given intravenously (0.25 to 0.5 mgm.). Relief was somewhat less sure if the subcutaneous route was used. Oral administration (1 to 2 mgm.) was of value only in mild attacks. Relief required about 30 minutes by the intravenous, 60 minutes by the subcutaneous, and two to three hours by the oral route. Eight of the patients have used ergotamine tartrate with repeated attacks for a period of from 6 to 18 months. In one of these, the effectiveness of the drug has been lost and its use discontinued. In the other seven patients, individual attacks are uniformly relieved. The attacks are less frequent in two patients, more frequent in three, and have not changed in two.

Ergotamine is not ordinarily effective in headaches which are not migrainous in character. Investigations are being conducted in an effort to explain the mechanism by which ergotamine tartrate so often produces such dramatic relief.

The Circulatory Response to Exercise in Patients with Angina Pectoris. Therapeutic Implications. By S. H. PROGER and (by invitation) W. R. MINNICH, Boston, Mass.

The circulatory response to exercise has been studied in 16 patients with angina pectoris and 6 normal controls in the same age group. A stationary bicycle at a fixed load and speed was used. Blood pressure, pulse rate, respiratory rate, pulmonary ventilation and oxygen consumption were recorded before, during and after exercise.

There were four general types of response, the most significant disturbance in these groups being: (1) failure of pulse rate to rise normally during exercise; (2) primary respiratory distress with rapid pulse rate; (3) the development of extrasystoles shortly before the onset of pain and disappearance shortly after cessation of exercise; (4) a rapid, barely palpable pulse during exercise which becomes strong after cessation of exercise. In only one patient could the response be termed normal.

Groups 1, 2 and 3 suggest definite corrective therapeutic procedures, which have been studied. Quinidin was strikingly effective in Groups 1 and 3.

The effects of thyroidectomy, cold temperature, a large meal and nitroglycerin were also studied.

Arteriography. By EDGAR V. ALLEN and (by invitation) JOHN D. CAMP, Rochester, Minn.

The arteries of the upper extremity of 75 living subjects were visualized roentgenographically following intra-arterial injection of a radiopaque medium. Local anesthesia was used and incision of the skin was unnecessary.

Three stages of involvement of arteries were seen in thrombo-angiitis obliterans: the primary stage was shown as simple changes in the contour of the artery; the secondary stage appeared as extensive encroachment on the arterial lumen which was represented by irregular channels; the tertiary stage was indicated by complete occlusion of the artery. "Patchy" involvement was characteristic; an artery might be extensively involved, while those in the immediate neighborhood appeared normal and all stages of involvement could be seen in the same arteriogram. The collateral circulation was impressive and appeared able to nourish the tissues of an extremity adequately when the main arteries were completely occluded. Arteries extensively sclerosed had an irregular, moth-eaten appearance and the lumens were reduced in caliber. An aneurysm of the popliteal artery showed clearly as a saccular dilatation surrounded by a mass of soft tissue representing mural thrombosis. Arteriovenous fistulas were characterized by enlargement of the arteries leading to the fistulas, "pooling" of the radiopaque medium in the region of the fistulas and incomplete filling of the arteries distal to the fistulas. The digital arteries of patients with scleroderma were frequently observed to be fine and twig-like; circulation to the digits was definitely impaired. Interpretation of the findings in scleroderma must await more extensive pathologic observations.

The Demonstration of a Parathyreotropic Substance in Increased Amounts in the Urine of Patients with Hyperparathyroidism Due to Diffuse Hyperplasia of All Parathyroid Glands. By SAUL HERTZ (introduced by Fuller Albright), Boston, Mass.

We have demonstrated elsewhere that anterior pituitary extracts contain a substance which regularly produces parathyroid hyperplasia in a rabbit. It was, therefore, of interest to see whether a parathyreotropic substance could be found in the urine of the two patients with clinical hyperparathyroidism in whom hyperplasia of the parathyroid glands rather than adenoma was found. This we have succeeded in doing. The gross and histological findings in the parathyroid glands of the injected rabbits are presented.

This finding establishes, we believe, on a firm foundation the existence of a disease entity due to the overproduction of a parathyreotropic substance. That this substance in these cases originates in the pituitary is an attractive but as yet unproven hypothesis.

The Phosphatase Content of Blood Plasma and Tumor Tissue in Malignant Diseases of Bone. By CLIFFORD C. FRANSEEN (by invitation) and JOSEPH C. AUB, Boston, Mass.

Determinations of the phosphatase content of blood plasma by Kay's method, show a distinct elevation in patients bearing an osteogenic sarcoma. After removal of the osteogenic lesion, as by amputation, the level of the plasma

phosphatase rapidly returns to normal; but with recurrence of the tumor, whether locally or as metastases, the plasma level again rises. The phosphatase content of osteogenic tissue exceeds that of any other tissue examined. Correlation of the phosphatase content of various portions of these tumors with the microscopic findings suggests that the enzyme is the product of the osteoblast, or of its immediate precursor, since it is present in large quantities even before osteoid tissue is produced and while the primitive osteoblast still resembles the fibroblast. Elevation of the level of blood plasma phosphatase may reveal early metastases or recurrences in patients who have had primary osteogenic sarcomata removed. Investigations indicate that the determination of the phosphatase level of the blood and the phosphatase content of the tumor tissue removed at operation may be of clinical value in the differential diagnosis of bone tumors.

Factors Determining the Effect of Exercise on Blood Sugar in the Diabetic. By RUSSELL RICHARDSON, (introduced by J. H. Austin) Philadelphia, Pa.

Sixty diabetic patients, without food or insulin for 16 hours, were given a standardized form of exercise on a rowing machine, blood sugars being taken at the beginning and at the end of the half-hour period of exercise. The blood sugar decreased during exercise in patients whose fasting blood sugar was below 175, remained constant in those with a fasting blood sugar between 175 and 300 and usually increased in those patients with a fasting blood sugar above 300.

On eight from the last group the experiment was repeated but with 0.1 unit of insulin intravenously; seven of these then showed a decreased blood sugar during the exercise.

Two patients with severe diabetes were given repeated exercise with different combinations of insulin and food. It was found that exercise after insulin or food is accompanied by a fall in blood sugar in patients in whom the same exercise, during the fasting state, results in an unchanged or increased blood sugar.

The results after insulin with rest and with exercise, and after rest or exercise without insulin were investigated in a group of 10 diabetic patients of various degrees of severity. These experiments showed that 0.5 unit of insulin given intravenously was apparently used up during the succeeding half hour of rest and was not available during the immediately following half-hour period of exercise.

The effect of exercise in the diabetic depends upon the initial level of blood sugar and is influenced by even very small amounts of effective insulin.

Cost of Work in Patients with Hypermetabolism Due to Leukemia and to Graves' Disease. By STELLA PAISLEY BRIARD and J. T. McCLINTOCK (by invitation) and C. W. BALDRIDGE, Iowa City, Iowa.

The metabolic rate in the standing position and the cost of horizontal walking in gram calories per kilogram meter were measured in 12 patients with leukemia and in 8 patients with Graves' disease.

In the patients with leukemia the basal metabolic rate varied from 4 to 78 per cent above normal (average 35 per cent). The cost of walking varied from 14.7 per cent above normal to 34.4 per cent below normal (average of 28 tests: — 5.8 per cent). The average standing metabolism was 15.4 per cent above the basal metabolic rate (normal about 12 per cent). Of the energy required for walking, 27.5 per cent was used to lift the body.

In the patients with Graves' disease the basal metabolic rate varied from 7 to 72 per cent above normal (average 40 per cent). The cost of walking varied from 4.6 to 73.1 per cent above normal (average of 20 tests: + 38.5 per cent). The average standing metabolism was 25.1 per cent above the basal metabolic rate. Only 15.5 per cent of the energy used in walking was used to lift the body. One patient with myxedema (basal metabolic rate: — 42 per cent) used 18.6 per cent less energy than normal in walking. The standing metabolism was used as the base from which to calculate the cost of walking.

Abdominal Disease Simulating Coronary Occlusion. By PAUL S. BARKER, FRANK N. WILSON and (by invitation) FREDERICK A. COLLER, Ann Arbor, Mich.

In recent years much emphasis has been given to the fact that acute coronary occlusion may resemble acute surgical conditions of the upper abdomen. The fact that disease of the upper abdomen may simulate coronary occlusion has been frequently overlooked. Owing to this, and to the widespread interest in coronary occlusion, cases of upper abdominal disease are now encountered in which the erroneous diagnosis of coronary occlusion or angina pectoris has' been made. Unless the symptoms and signs are unequivocal, the diagnosis of angina pectoris or coronary occlusion should not be made until upper abdominal disease has been excluded. Five illustrative cases are presented. In the first three the symptoms were caused by gallbladder disease, but erroneous diagnoses of coronary disease had been made. The fourth was a case in which perforation of a gastric ulcer was accompanied by many of the manifestations of acute coronary occlusion, and only rather complete studies revealed the true condition. In the fifth case both cholelithiasis and angina pectoris were present. Coronary occlusion occurred a few days after cholecystectomy. Following his recovery the patient had no cardiac or abdominal symptoms.

Intra-articular Changes Resulting from Displacement of the Patella. By GRANVILLE A. BENNETT (by invitation) and WALTER BAUER, Boston, Mass.

Information concerning the effects of unusual and continued trauma to joint structures is desirable for a better understanding of the factors involved in the production of hypertrophic or degenerative arthritis.

In an earlier investigation concerning the repair of articular cartilage in the knee joints of dogs, the patellae in a number of joints became permanently displaced. Such displacement was toward the medial side of the joint so that the patella was in apposition with the medial epicondylar ridge of the femur. This antatomical derangement was invariably followed by marked intraarticular changes similar to those of hypertrophic or degenerative arthritis in man. Because of these observations further studies concerning the effects of patellar displacement were undertaken. Such experiments were carried out on rabbits where the desired derangement can be produced without opening the joint space or otherwise injuring any of the joint structures. Gross and microscopic examination of these joints revealed that anatomical changes, similar to those of hypertrophic arthritis, occurred as early as four weeks. These changes progressed and became more marked in the experiments of longer duration. Marked lipping of the articular margins of the femur occurred. This was accompanied by degeneration of original articular cartilage surfaces and in some instances by eburnation of the denuded subchondral bone.

Evidence has also been obtained that hypertrophic or degenerative arthritis develops at an early age in human subjects with displaced patellae and that in

older individuals this anatomical derangement is apparently a cause of unusually extensive changes.

These experimental and clinical findings serve to emphasize the importance of continued trauma, unusual use, and wear and tear in the production of the intra-articular changes which have been designated as hypertrophic or degenerative arthritis.

The Effect of Elevated Metabolism on the Heart Weight of Frizzle Fowl. By ERNST P. BOAS and (by invitation) WALTER LANDAUER, New York, N. Y.

Frizzle fowl, owing to its scanty plumage, exhibits a profound disturbance of heat regulation with a resulting high basal metabolism in the absence of hyperthyroidism. We have shown that frizzle hens have heart rates 27 per cent, and frizzle roosters 68 per cent above that of normal chickens. We have weighed the hearts of 110 chickens by a modification of Müller's method. Of these 37 were normal females, 33 frizzle females, 20 normal males, and 20 frizzle males. In proportion to body weight the total ventricular weight of frizzle chickens is significantly greater than that of normal chickens. In frizzle pullets the difference is 0.7 with an error of ± 0.14. The ratios between right and left ventricles, whether or not the septum be included, are practically the same for frizzle as for normal fowl, so that both ventricles share equally in the hypertrophy that takes place. It would appear that hypertrophy, as well as tachycardia, are conditioned only by the high metabolism of the frizzle fowl. The fact that an increased metabolism alone may cause cardiac hypertrophy and tachycardia suggests that in Graves' disease the elevated metabolism per se increases the work of the heart, and in this manner directly contributes to the cardiac disturbances that are so commonly observed.

The Heredity Factor in Obesity: A Preliminary Report. By RAMSDELL GURNEY (introduced by Byron D. Bowen), Buffalo, N. Y.

Eighty-one per cent of 50 obese women questioned, were found to have one or both parents obese, as compared to 47 per cent of 50 non-obese women taken at random. The two groups were approximately from the same age periods. They had also approximately the same incidence of operations and pregnancies, these two being the most common factors associated with the onset of obesity— 13 and 46 per cent respectively.

The builds of the offspring of the different matings were also studied with results similar to those found by Davenport in his report in 1923. Difference in variability of the progeny of different matings was present with the offspring of two slender parents least variable and the offspring of the stout and slender matings most variable. These findings strongly support the presence of segregation which in turn is evidence for Mendelian inheritance. Obesity was only slightly dominant over slenderness.

Transmission of Encephalitis to Mice from Human Brain Material preserved three months in Glycerin and Comparison of this Virus with the Virus of an Epidemic of Trachcobronchitis and Pox present in Fowl in the St. Louis Area simultaneously with the Encephalitis Epidemic. By G. O. BROUN and (by invitation) R. O. MUETHER and W. D. COLLIER, St. Louis, Mo.

Brain material of two of seven cases of epidemic encephalitis preserved approximately three months in glycerin, yielded a virus pathogenic for white

mice and apparently identical with a strain of encephalitic virus secured through the courtesy of Dr. Muckenfuss.

Approximately, 75 per cent of the early cases in the St. Louis encephalitis had some contact with chickens. Moreover, tracheobronchitis and fowl pox was at that time present in epidemic proportions in many St. Louis county flocks including 20 flocks in close proximity to cases of encephalitis.

Chickens inoculated during the epidemic with fresh whole blood and brain material of encephalitic cases developed tracheobronchitis and pox. Spontaneous infection, however, can not be ruled out in these experiments.

The more recently isolated mouse virus has been compared with the virus of this epidemic disease of fowl.

While a mild, avirulent type of encephalitis has been produced in mice with material from chickens ill with pox and tracheobronchitis (acquired spontaneously or subsequent to inoculation with human brain material), nevertheless, the evidence at present available indicates rather distinct differences between the two viruses.

A Quantitative Study of Fat Absorption in Gallbladder Disease: Non-visualization of the Gallbladder and the Ability to Absorb Fat. By CHARLES L. BROWN and (by invitation) JOHN D. CAMERON, Ann Arbor, Mich.

This reports an investigation of the ability of patients with chronic cholecystic disease, without common bile duct obstruction, showing non-visualization of the gallbladder, to absorb fat.

Weighed mixed diets were given in two periods of three days each: the first, a control period, using 40 grams of fat daily; the second, a test period, using from 150 to 200 grams of fat daily. The diets for the two periods differed only in that additional fat, as butter, was given in the test period. The total feces for each dietary period was obtained, using charcoal and carmine as markers. Quantitative fat determinations on the feces were made by Saxon's method. Fractionation of the fat is in progress. Protein absorption was determined as a further control in this study.

Ten cases were studied. The coefficient of digestibility of fat was determined and found to range from 98 to 79 per cent, the average being 90 per cent. The normal coefficient of digestibility of butter fat is 97 per cent.

This study shows:

(1) Fat absorption may be normal even though there is non-visualization of the gallbladder.

(2) Cholecystic disease, without obstruction of the common bile duct, may be accompanied by diminished absorption of fat; an average in this series of cases is reported of 7 per cent, with one observation 18 per cent, below normal efficiency.

Antibody Response of Human Subjects to Intracutaneous Injections of Pneumococcus Carbohydrates. By MAXWELL FINLAND and HARRY F. DOWLING (introduced by William B. Castle), Boston, Mass.

Minute amounts of various carbohydrate fractions of Type I, II and III pneumococci were injected intracutaneously in normal individuals and in hospital patients free from recent infections. The soluble specific substances (Heidelberger, Goebel and Avery) and the cellular carbohydrates (Wadsworth and Brown) of virulent Type I, II and III strains and the cellular carbohydrates of a degraded, avirulent and non-type-specific strain derived from a virulent Type I strain were used. These were injected singly and in groups.

Single injections and repeated injections at intervals were used in different subjects. The blood of the subjects was tested before and at intervals after such injections for various immunological reactions to virulent Type I, II and III pneumococci. Type-specific opsonins, agglutinins and protective antibodies in varying degrees developed as a result of the injections. Only minor differences were observed between the serological immunity resulting from the soluble specific substances and that resulting from the cellular carbohydrates.

The Cerebral Circulation. XXXI. The Effect of Alcohol upon the Cerebral Vessels. By CAROLINE C. BEDELL (by invitation) and STANLEY COBB, Boston, Mass.

The effect of alcohol on the calibre of pial arteries was studied by the method described by Forbes[1]; a window was screwed into the skull of a cat and the arterial diameter measured microscopically with a micrometer eyepiece. Fifteen cats under Dial (" Ciba ") anesthesia were given 34 injections of 25 per cent alcohol; significant arterial dilatation resulted in 28 instances. The average dilatation was 19.5 per cent, reaching a maximum 4 minutes after starting the injection. In 62 per cent of the trials, a brief constriction averaging 7.1 per cent occurred one minute after the injection was started. The dilatation was roughly proportional to the size of dose and rate of injection, and quite independent of changes in systemic blood pressure. An increase in cerebrospinal fluid pressure was consistently noted, beginning shortly before the vessel under observation dilated. Simultaneous measurements of cerebral blood flow obtained with Gibb's flow recorder[2] gave added evidence of greatly increased cerebral circulation during the period of vasodilatation.

Intracarotid injection of alcohol in much smaller dosage produced an average dilatation of 35 per cent in 12 out of 13 trials, with little or no change in systemic blood pressure. Alcohol administered by stomach tube to anesthetized cats was not followed by vasodilatation, but in a cat observed after full recovery from anesthesia the artery dilated 16 per cent in 23 minutes, returning to normal 26 minutes later.

The Nature and Action of a Pressor Substance Found in Body Fluids of Man. By IRVINE H. PAGE (introduced by A. E. Cohn), New York, N. Y.

Extracts of human blood plasma, ascitic and cerebrospinal fluids contain a substance which has a powerful pressor action. The plasma colloids appear to hold it in a bound state. Coagulation alone of the blood does not cause the substance to be formed. Its action suggests that its pressor effect is brought about by mediation of the central nervous system. The rise in pressure of the test animal is due especially to constriction of the arteries in the splanchnic region.

Assay of the pressor extract is made difficult because of the dependence of the vascular response on the functional state of the central nervous system. No evidence has been obtained indicating that these substances are increased in amount in patients suffering from hypertension of varied pathogenesis. Extract of the ventricular and spinal fluids and urine of certain patients has much greater vaso-activity than normal.

The pressor action of extracts of human body fluids appear to be a phenomenon which is species-specific.

[1] Forbes, H. S., Arch. Neurol. and Psych., 1928, 19, 751.
[2] Gibbs, F. A., Proc. Soc. Exper. Biol. and Med., 1933, 31, 49.

The belief that in cases of nephritic hypertension some substance is present in plasma which sensitizes the blood vessels to epinephrin receives no support from studies on pithed cats.

A Photoelectric Method for the Measurement of the Number of Red Cells and Hemoglobin Concentration in the Same Small Sample of Blood. By RONALD V. CHRISTIE and (by invitation) KENNETH A. EVELYN, Montreal, Canada.

The apparatus is essentially a photoelectric opacity meter consisting of a light source, adjustable filter, absorption cell, photoelectric cell and galvanometer. The light source is a 2 c.p. 6 volt bulb in a parabolic reflector. The conventional rectangular glass absorption cell is replaced by a test tube which also serves as a cylindrical lens to focus the light on the photoelectric cell. A vacuum alkali cell is used for maximum stability.

A filter which transmits only the red end of the spectrum (in which region absorption by hemoglobin is negligible) is inserted, 0.05 cc. of blood is diluted to 10 cc. in the absorption tube and is further diluted until the opacity falls to a value which corresponds to a known concentration of erythrocytes of normal size. The erythrocyte concentration is calculated from the dilution required. The cell diameter is measured on a halometer attached to the apparatus and a correction appplied if necessary; the correction for size is negligible except in extreme cases.

The sample is then laked with saponin, the filter changed to transmit a band including both the main hemoglobin bands, and the hemoglobin concentration is measured as with any other photoelectric hemoglobinometer.

Errors in the erythrocyte count are ± 5 per cent, in the hemoglobin concentration ± 2 per cent. The technique is simple, the apparatus inexpensive and the entire determination requires 4 minutes.

Splenectomy for Acute Hemoclastic Crises. By CHARLES A. DOAN (by invitation), GEORGE M. CURTIS and BRUCE K. WISEMAN (by invitation), Columbus, Ohio.

Splenectomy, performed in patients with hemolytic jaundice, is followed at once by a definite increase in the erythrocytes and hemoglobin. This ensues immediately after operation and amounts, essentially, to a spontaneous autotransfusion. Investigations made during the past three years on twenty-four clinical cases occurring in eight families have resulted in therapeutic splenectomy in eight individuals. Prompt relief from the principal symptoms of the disease ensued in each instance. It appeared that the spleen was the main etiologic factor in the production of the hemolytic syndrome.

Acute hemoclastic crises occur at intervals in the course of the disease. They may be precipitated by various causes, such as fracture or other injury, intercurrent disease or by major surgery. It is the usual teaching that splenectomy should not be carried out during a crisis. On the other hand, our investigations have led us to conclude otherwise. Splenectomy has been performed four times during spontaneous or induced acute hemoclastic crises. One of these patients was a four year old girl with 800,000 red cells and 100 per cent reticulocytes. In each of these patients, the characteristic autotransfusion ensued immediately after the splenectomy, with complete recovery following in due course.

Observations on the Autonomic Control of Cardiac Vasculature. Attempted Vagus and Sympathetic Overaction on Cat's Heart. By HAROLD F. ROB-ERTSON and ARTHUR J. DERBYSHIRE (by invitation) and ELLIOTT C. CUT-LER, Boston, Mass.

In some cats unilateral or bilateral division of the sympathetic or parasympathetic innervation to the heart or of both was carried out. In other cats unilateral or bilateral suture of the cephalic end of the phrenic nerve to the caudal end of either the cervical sympathetic trunk or the vagus nerve was done, the opposing innervation to the heart being cut in a few of these cases. Animals were examined for changes in cardiac function and in cardiac response to drugs. Anastomosed nerves were tested periodically with an amplifier and loudspeaker to ascertain whether typical phrenic impulses were carried caudad from the site of suture and to what location. At the same time the heart could usually be inspected. As occasion arose, autopsies were done and sections made to study the cardiac vessels and to determine the courses and terminations of the anastomosed nerves.

Neither denervation nor nerve suture has produced, after many months, any cardiac change. Electrical evidence, checked histologically, has demonstrated growth of anastomosed nerves toward the heart. There has been no functional or anatomic evidence that autonomic end-organ or synaptic anastomoses with the down-growing phrenic fibers have taken place.

Some Responses of Normal Kidneys to the Intravenous Injection of Parathyroid Extract. By READ ELLSWORTH and (by invitation) JOHN EAGER HOW-ARD, Baltimore, Md.

Individuals in whom the kidneys gave evidence of functioning normally were kept fasting, at rest, and on a given fluid intake. Urine was collected hourly for three hours before and three hours after the intravenous injection of 40 units of parathormone. Following the extract there was usually, but not always, a diuresis. There was no alteration in urea clearance. There was a tendency for the pH to shift toward the alkaline side. The chloride excretion usually rose very slightly in the first hour after the injection and then decreased quite markedly in the second and third hours. Most striking was the outpouring of phosphate in the urine. This was very conspicuous during all three hours after the parathormone. The phosphate clearance in cc. plasma cleared per hour increased from two to five-fold after the injection.

The phosphorus diuresis was accompanied by a relatively slight fall in serum inorganic phosphate.

A definite effect of intravenous parathyroid extract upon the normal kidney is indicated, most conspicuously apparent in the excretion of phosphate. It is similar to the effect of intramuscular injection in hypoparathyroid patients previously reported.

The Effect of Gastrectomy on the Assimilation of Food. By EDWARD S. EMERY, JR., Boston, Mass.

This study was stimulated by observing a patient who developed a steatorrhea following a subtotal gastrectomy. The object of the investigation was to determine the effect of removal of the stomach on the assimilation of food and to investigate the reasons for any failure of assimilation. The method of study consisted of feeding dogs weighed amounts of food and comparing the amounts of carbohydrate, protein and fat ingested with the amounts lost through the

stools. A control series was obtained on five normal dogs, and studies were made on two dogs with a complete gastrectomy. The outstanding finding was a markedly deficient utilization of fat. The utilization of nitrogen was only slightly impaired, and carbohydrates were assimilated normally. Utilization of fat was not improved by the administration of hydrochloric acid, raw pancreas and pancreatic ferments, bile salts or fatty acids. It was definitely improved by small feedings at frequent intervals. The data suggest that removal of the stomach interferes with the utilization of fat because of the loss of the stomach's function of distributing food to the intestine according to the physiological requirements of the latter.

Observations on a Case of Paroxysmal Hemoglobinuria. By A. CARLTON ERNSTENE and (by invitation) W. JAMES GARDNER, Cleveland, Ohio.

A study has been made of a case of paroxysmal hemoglobinuria in a man who presented no clinical or laboratory evidence of syphilis. The Donath and Landsteiner reaction for the presence of hemolysin in the blood serum was consistently positive. Spontaneous attacks of hemoglobinuria occurred after exposure to temperatures as high as 52° F. and were accompanied by deep cyanosis of the fingers, toes, nose and ears. Hemoglobinuria could be produced regularly by the application of ice packs from the feet to the level of the anterior superior spine of the ilium or to the xiphoid process for twenty minutes or longer. After spinal anesthesia extending to the nipple line, however, ice packs did not cause hemoglobinuria, nor did hemoglobinuria occur when spinal anesthesia was followed by exposure of the entire body to a temperature of 51° F. for one hour. Injection of novocaine into both lumbar sympathetic chains did not prevent the production of hemoglobinuria by ice packs.

In view of these observations, it was decided to resect the left splanchnic nerves and remove a portion of the left lumbar sympathetic chain. The patient remained in the hospital for three weeks after the operation, and during this time several attempts to induce hemoglobinuria with ice packs failed without exception. The Donath and Landsteiner reaction was repeated on the fifteenth, eighteenth and twenty-fifth days after operation and was negative on each occasion. Soon after discharge from the hospital, the patient reported having passed a single specimen of red urine after remaining out-of-doors for nearly three hours at a temperature of 18° F. Attempts to produce hemoglobinuria with ice packs were unsuccessful at that time. One month later, however, ice packs regularly induced attacks. A second operation, therefore, was performed in which the right splanchnic nerves were severed and the second and third lumbar sympathetic ganglia removed. Subsequent attempts to produce hemoglobinuria with ice packs have failed. The Donath and Landsteiner reaction has remained negative since the first operation. The observations suggest that paroxysmal hemoglobinuria is due fundamentally to a disturbance of the sympathetic system, at least in cases not associated with syphilis. They also indicate a promising approach to the treatment of the condition.

The Continued Administration of Large Amounts of Irradiated Ergosterol to Patients Suffering from Postoperative Parathyroid Tetany: Its Therapeutic Value and Effect on Calcium and Phosphorus Metabolism. By R. F. FARQUHARSON, Toronto, Ont.

A number of patients suffering from post operative parathyroid tetany have been treated with very large doses of irradiated ergosterol for prolonged periods during the past two years. In all cases over periods of from three to four

weeks, the serum calcium could be raised to normal, the urinary excretion of calcium increased, and the tentany relieved by the oral administration of a concentrated solution of irradiated ergosterol in amounts equivalent to 20 to 60 cc. of commercial viosterol daily. The effect of such administration might last for one month or more after cessation of therapy. In one case, after almost continuous administration of large doses for nearly two years, the continued ingestion of a much larger dose failed to relieve tetany completely or to increase the serum calcium materially.

The effect of such prolonged treatment on the serum calcium and phosphorus and the excretion of calcium and phosphorus in the stools and urine of a control patient as well as of patients with parathyroid tetany is reported.

Fusospirochetal Pneumonia. By HENRY FIELD, JR., Ann Arbor, Mich.

The importance of the fusospirochetal group of organisms in chronic lung diseases, particularly lung abscess and bronchiectasis, has become increasingly apparent. While it has been recognized that these end states are preceded by a pneumonic stage, fusospirochetal pneumonia has not been adequately recognized and described. Our observations lead us to believe that it is comparatively common and that it has distinctive features which make it a clinical entity.

Although sometimes fairly acute and severe, it is commonly subacute to chronic, with mild fever and little cough or sputum in the presence of extensive consolidation. The sputum is commonly not foul. The x-ray appearance is somewhat characteristic, particularly the slow progression and regression of consolidation which may not correspond with the course of symptoms. There is usually complete or nearly complete resolution of consolidation and symptomatic cure, although asymptomatic bronchiectasis or abscess is a frequent residual. Bronchiectasis has been demonstrated by lipiodal instillation six weeks after onset, and nearly complete resolution, without appreciable bronchiectasis, has occurred after nine months.

The effect of arsphenamine treatment has been carefully observed. The fusospirochetal organisms have persisted in the sputum following its administration in intensive doses. A spread of consolidation and fusospirochetal gingivitis have been observed soon after its administration. Its effectiveness needs further confirmation.

A Study of Fat Tolerance Tests. By HARRY BLOTNER (by invitation) and REGINALD FITZ, Boston, Mass.

A group of patients has been given an oral test meal of 500 cc. of 20 per cent fat and the blood cholesterol has been estimated at two-hourly intervals thereafter for eight hours. Certain bizarre results of some interest have been obtained. The normal individual's blood fat remains essentially constant. An obese person's or a person's with diabetes insipidus shows a progressive rise in the blood cholesterol concentration and this rise can be sharply inhibited by the use of posterior lobe pituitary extract. Insulin appears to cause a rise in the concentration of blood cholesterol in normal individuals receiving insulin as a means to induce gain of weight. These observations add further evidence to demonstrate the antagonistic effect of insulin and posterior lobe pituitary extract and they suggest further that obesity may be definitely related to the pituitary gland and that in certain cases obesity has an endocrine background as its basis.

Changes in Specific Gravity, Total Nitrogen, and Colloid Osmotic Pressure of the Plasma in Normal and Edematous Dogs following Salyrgan. By MARSHALL N. FULTON and (by invitation) A. H. BRYAN, WILLIAM EVANS, JR., and E. A. STEAD, JR., Boston, Mass.

Studies have been made in normal dogs following the administration of water which indicate that measurements of the specific gravity, total nitrogen, and colloid osmotic pressure of the plasma may be used as an index of dilution or concentration of the blood. In search of evidence of the extrarenal action of salyrgan, similar measurements were made in normal dogs after administering salyrgan intravenously. To secure a much greater diuretic response, the animals were then made edematous by plasmapheresis plus the feeding of salt. Under these conditions, salyrgan causes a diuresis 15 to 20 times as great as that observed in normal dogs. The urine flow may amount to as much as 11 cc. per minute for a half hour period which is comparable to 50 or 60 cc. per minute in man—a diuresis which should be suitable to furnish evidence of an extrarenal action of this drug. Measurements made each hour during such a diuresis of the specific gravity and nitrogen concentration of the plasma and at various intervals of the colloid osmotic pressure of the plasma do not indicate any dilution of the blood preceding the diuresis. On the contrary, during the course of the diuresis and apparently as a result of it, there occurred measurable changes in these factors which indicate an actual blood concentration. Such findings suggest a renal effect as the chief if not the sole cause of diuresis following salyrgan.

The Value of Abdominal Compression in the Treatment of Chronic Pulmonary Tuberculosis with Cavitation. By BURGESS L. GORDON, Philadelphia, Pa.

It was shown in previous studies that elevation of the diaphragm due to abdominal tumors decreased the extent and activity of tuberculous lesions. This suggested the use of abdominal compression for the treatment of tuberculosis. Special abdominal supports were devised and the indications studied. Roentgenograms of the lungs show a marked decrease in cavitation in fibroid cases; improvement in cough, dyspnea and expectoration were striking. The mechanism has been considered.

Clinical and Pathological Observations on the Heart in Trichinosis. By WESLEY W. SPINK (introduced by Clark W. Heath), Boston, Mass.

Two cases of severe trichinosis were studied with special attention to a complicating myocarditis. The first patient entered the hospital with the typical clinical picture of trichinosis. Examination of the heart revealed a gallop rhythm and tachycardia. Arterial hypotension also occurred. On the sixteenth day of the disease, the electrocardiogram showed T-1 and T-3 inverted and T-2 inverted with upward convexity. The same changes were present four days later. Four weeks later the electrocardiogram was interpreted as normal.

The second patient died forty-eight hours after he was first seen. At postmortem examination death was attributed to a severe myocarditis and bronchopneumonia. Microscopic examination of sections of heart muscle revealed no larvae. After peptic digestion of the whole heart, fourteen larvae were found in the sediment. They were of the size usually found in skeletal muscle and not the smaller forms found in the circulating blood, thus excluding the possibility of their presence in the blood vessels of the heart.

Doubt exists in the medical literature as to whether the myocarditis of trichinosis is due to the presence of the parasite or to blood-borne "toxins."

Clinical reports have not included electrocardiographic observations. The myocarditis of trichinosis may result from an invasion of the muscle by the parasite. The lesion occurs as early as the second week of the disease as observed in electrocardiographic and clinical studies, and may be the cause of death in the sixth week.

The Analgesic Effect of Jaundice on the Rheumatic State. By PHILIP S. HENCH, Rochester, Minn.

About twenty-five patients with different kinds of "chronic rheumatism" (arthritis, fibrositis, sciatica, sciatic pain) have been seen who have developed intercurrent jaundice. During the jaundice and for variable periods thereafter the majority of patients were "completely cured," and there was either complete or marked amelioration, not only of pain, but of stiffness and soreness, and in some cases of swelling. This paper presents the clinical data on these cases, the types of jaundice concerned, the relationship between the serum bilirubin and the van den Bergh and the degree of analgesia experienced, considerations of the mechanisms involved, and experimental attempts to repeat the analgesic effect.

The Peripheral Vascular Effects of Freezing and the Amelioration by an Intermittent Negative Pressure Environment. By LOUIS G. HERRMANN (by invitation), and GEORGE HERRMANN, Galveston, Texas.

The pathologic physiology of the circulation of blood in the ears of a series of hares after various degrees of freezing caused by the application of carbon dioxide snow, liquid air, ethyl chloride spray or natural cold, was studied in detail. The early constrictor effect of cold was found to be of short duration. Curves showing this effect will be demonstrated. The second or important stage, i.e. capillary stasis, was likewise studied. When capillary stasis was permitted to remain unchanged for several hours, disintegration of the cellular elements of the blood took place and the blood in the arterioles, capillaries and venules coagulated. In the untreated animals gangrene resulted. At the stage of capillary stasis in another series of animals, the affected ears were treated by rhythmic alternation of the environmental pressure from about 50 mm. of mercury *negative* pressure to about 20 mm. of *positive* pressure at the rate of 4 cycles per minute. A special treatment jar of glass was constructed in order that one ear could be treated and observations be made without influencing the circulation of other parts of the animal's body. Restoration of the arterial circulation resulted after 20 minutes of such treatment and gangrene was prevented. A clinical case of bilateral frozen feet was treated in this manner and within 12 hours the arterial circulation was reestablished in both feet. Only slight necrosis of the skin of four toes resulted. Photographs and charts will illustrate the experimental and clinical basis for this form of therapy.

Acute Lead Poisoning Experimentally Produced. By BAYARD T. HORTON and (by invitation) J. ARNOLD BARGEN, and ARNOLD E. OSTERBERG, Rochester, Minn.

During the past three years, while attempting to treat with lead subjects with inoperable cancer, we have had an unusual opportunity to observe the development of early signs and symptoms of acute lead poisoning. Eighty-one subjects received 476 intravenous injections of either colloidal lead phosphate or lead selenide. Each subject received an average total dose of 440 mgm. of lead. Immediately, fever, chills, generalized aches and pains, nausea or vomit-

ing occurred in 101 instances, 21 per cent). These symptoms promptly disappeared. Approximately two weeks after completion of the treatment, 44 subjects developed signs and symptoms of acute lead intoxication which consisted of general malaise, weakness, headaches, generalized aches, shooting pains in the extremities, abdominal cramps, nausea and vomiting, blue gums and sallow complexion. These features persisted from two weeks to three months. Wrist drop and toe drop were observed only in one subject. Patients varied markedly in their tolerance to lead as illustrated by its effect on the erythrocytes and its excretion in the urine. When lead was excreted in the urine, it was in small amounts (0.1 mgm. to 0.2 mgm. each day) with no relationship to the amount previously administered. The spleen and liver were rendered roentgenologically visible.

Inadequate Amount of Gastric Secretion as a Factor in the Production of Pernicious Anemia. By RAPHAEL ISAACS and S. M. GOLDHAMER (by invitation) and C. C. STURGIS, Ann Arbor, Mich.

The average amount of secretion of gastric juice, with or without histamin injection, in 63 collections in 10 patients with pernicious anemia was 20 cc. per hour as compared with about 150 cc. per hour in normal individuals under the same conditions. Gastric secretion collected from untreated patients with pernicious anemia was incubated with ground beef. This, in amounts comparable to that secreted by normal individuals, was then fed daily to two patients with pernicious anemia. Data on the blood changes which followed are given. The response suggests that the gastric secretion in pernicious anemia may have the precursor of the hematopoetic stimulant, but, because of the defective amount secreted, may not be able to raise the blood count above a certain minimal level.

The Characteristics of the Synovial Fluid in Gonococcal Arthritis. By WALTER K. MYERS and WILLIAM F. HOLMES, JR. (by invitation) and CHESTER S. KEEFER, Boston, Mass.

In order to gather more precise information regarding the characteristics of the synovial fluid in gonococcal arthritis, 54 samples from 40 patients were studied. Total and differential leukocyte counts were made on the fluid, and the total protein, sugar and nonprotein nitrogen content of the fluid was compared with that of the blood. Gonococcal complement fixation tests and bacterial cultures were made at the same time. The fluids were divided into two groups—the uninfected and the infected. The results were as follows:

The total leukocyte count was somewhat higher in the infected fluids; the polymorphonuclear cells were present in greater numbers and the monocytes and clasmatocytes in fewer numbers than in the uninfected fluids.

The total protein was increased in both, varying from 3.5 to 6.0 per cent. The nonprotein nitrogen content was the same in the blood and synovial fluid. The sugar content of the fluid depended upon three factors: the level of the blood sugar, the number of leukocytes and the presence of organisms; of these, the first two were more important than the third.

Gonococcal complement fixation reactions were the same in the joint fluid as in the blood serum, and were positive in 74 per cent of the cases.

The examinations of most value were the total and differential counts, and the bacteriological and serological examinations. Chemical examinations yielded very little of either diagnostic or prognostic value.

The Effect on the Heart of Experimental Pleural Conglutination. By HORACE M. KORNS and (by invitation) HARRY LANDT, O. R. HYNDMAN, RAYMOND GREGORY, and CLARK N. COOPER, Iowa City, Iowa.

Revival of the Human Heart. By W. B. KOUNTZ and (by invitation), M. PRINZMETAL, St. Louis, Mo.

A method by which the human heart may be revived soon after death has been applied to sixty-two bodies. Moving pictures showing details of the method are presented.

The important feature of the apparatus is that it maintains a constant pressure relationship in the ventricle and in the coronary system. Details are shown from the initial autopsy incision to the demonstration of the beating heart together with electrocardiographic tracings taken at intervals during the period of revival. This method permits the study of a number of physiological problems which can be attacked by no other means. The right and left bundle branches of the revived heart have been cut and extrasystoles have been produced at different points. A record of the results will be shown.

Recurrent Agranulocytosis. By DORAN J. STEPHENS (by invitation) and JOHN S. LAWRENCE, Rochester, N. Y.

Detailed observations have been made over a period of eighteen months in the case of a 47-year old woman who has had thirteen attacks of recurrent granulocytopenia. During the first five months of observation there were five periods of neutropenia three of which were accompanied by infection of the upper respiratory tract. Each period of neutropenia coincided with a menstrual period. Sternal biopsy showed an essentially normal bone marrow. Bilateral oophorectomy was done, following which there was a modification of the neutropenic cycle. Estimations of urinary excretion of female sex hormone were made before and after operation. The administration of theelin was without apparent effect on the neutropenic cycle. Satisfactory remissions of individual attacks of granulocytopenia were observed with and without the use of pentnucleotide.

In control experiments, white blood cell counts and differential counts of six normal young women were studied over a period of two months. There was no significant change in the white blood cell picture at the time of the menstrual period.

The Calorigenic Action of the Optically Active Isomers of Thyroxin. By J. LERMAN and W. T. SALTER, Boston, Mass.

For many years it has been known that pure racemic thyroxin is less active than crude thyroid extract in the same thyroxin iodine dosage. Optical activity and peptide linkage have been suggested as explanations. In a previous report to this Society, natural thyroxin polypeptide was shown to have only the activity of racemic thyroxin and of whole gland administered in equi-iodine dosage. Since then, both d- and l- thyroxin (donated by Harington) have been tested in five patients with myxedema and found to yield identical results when administered intravenously in daily rations. The calorigenic responses check the standard polypeptide curve, previously reported.

In normal rats, l-thyroxin has been shown by other authors to be three times as active as the d- isomer. It is inferred that the relatively large dosage employed in such experiments constituted a pharmacological observation rather

than a physiological effect. Moreover these experiments were conducted on animals possessing intact thyroid glands—a condition which prevents the proper evaluation of results.

The present data substantiate the previous inference that thyroxin is not the only active constituent of thyroglobulin.

The Action of Injected and Secreted Adrenalin Before and After Total Thyroidectomy. By MORTON G. BROWN and MARGARET M. SAWYER (by invitation) and SAMUEL A. LEVINE, Boston, Mass.

The sensitivity to adrenalin as indicated by the rate of the denervated heart was studied in two groups of cats. In the first group of cats the hearts were denervated. The adrenals were then stimulated in two ways. First, the cats were put in a cold room at 1° C. for 45 to 60 minutes. Secondly, the cats were made to run on a treadmill at a constant rate for a given length of time. The increase in heart rate caused by this stimulation was noted. Total thyroidectomy was then performed and it was found that the rate response to these stimuli was only about 50 per cent of the preoperative response. The preoperative response could be restored by increasing the metabolism by feeding thyroxin.

In the second group of cats the adrenals were inactivated as well as having the hearts denervated. A given amount of adrenalin of standard strength was then injected intravenously and the increase in heart rate was noted. These cats then had a total thyroidectomy and it was found that the rate response to the same amount of adrenalin was only about 50 per cent of the preoperative level and could be restored by increasing the metabolism by feeding thyroxin.

We believe that these observations throw some light on the clinical results obtained from total thyroidectomy in the treatment of intractable heart disease.

The Heart in Scarlet Fever. By JAMES M. FAULKNER (introduced by E. A. Locke), Boston, Mass.

The object of the study was to determine the incidence and character of the cardiac involvement in scarlet fever.

A total of 171 cases of scarlet fever were studied during the acute illness. In view of the unreliability of auscultatory findings in the heart in acute infections particular importance was placed on the electrocardiogram. One or more electrocardiograms were taken in each case. Electrocardiographic changes observed were prolonged P–R interval in five cases and T-wave changes in five cases. The number of cases showing electrocardiographic changes was ten or 5.8 per cent. In addition, nine cases exhibited a P–R interval of 0.20 second, a finding of borderline significance. Cases which developed scarlatinal arthritis showed an increased tendency to electrocardiographic abnormalities. Of 142 cases of scarlet fever without arthritis, eight or 5.6 per cent showed such changes, whereas in twenty-nine cases with arthritis, two or 7 per cent showed them. The difference is, of course, too small to be significant but if we include cases showing a P–R interval of 0.20 second, it becomes more striking, being 9.8 per cent for cases without arthritis and 17.2 per cent for cases *with* arthritis.

In addition, a follow-up study was made of 600 cases of scarlet fever *from* one to three years after the acute infection. Of these 600 cases, seven had developed signs of heart disease. In two, the signs of endocarditis had appeared while the patients were still in quarantine. In the third, a typical attack of rheumatic fever occurred immediately after discharge from quarantine. An-

other suffered from three attacks of rheumatic fever during the interval between the scarlet fever and the follow-up examination. In the remaining three cases, the physical signs of valvular disease were found without any intercedent history of rheumatic fever or chorea. There appeared to be no relationship between the severity of the infection and the subsequnt development of heart disease. None of this group had scarlatinal arthritis. The type of heart developed in these cases was indistinguishable clinically from "rheumatic" heart disease.

The conclusion to be drawn from these observations is that scarlet fever is occasionally the inciting factor in the "rheumatic state." The incidence of heart disease following scarlet fever is small and perhaps no greater than that following simple acute tonsillitis.

The Relation of Fat Oxidation to Phosphocreatine Metabolism and Creatinuria. By ROBERT O. LOEBEL, New York, N. Y.

In the light of recent work one might expect a close relation to exist between the muscular weakness of diabetes or of carbohydrate deficiency on the one hand and the creatine phosphate metabolism and creatinuria on the other.

In this study four epileptic patients and one diabetic on creatine-free diets were studied for a period of 1 to 3 months; ketosis being induced in the former by high fat diets and fasting, in the latter by the withdrawal of insulin. Ketonuria appeared before increased amounts of creatine were excreted. The amounts of the latter were much smaller than has been observed (under somewhat different conditions) by previous workers who did not distill off the ketone bodies before determining the creatinine.

The ketonuria produced little or no change in creatine tolerance (the amount of ingested creatine which is retained and not excreted).

To interpret these results the synthesis of phosphocreatine (creatine $+$ phosphate $\leftrightarrows$ phosphocreatine) was directly measured in the excised muscle of normal and diabetic dogs. In 4 normal dogs the phosphocreatine of the excised muscle was reduced by 20 minutes of nitrogen exposure to an average value of 8.9 mgm. P per 100 grams of muscle. When the muscle was allowed to respire for 2 hours at 37.5° C. in pure oxygen in a solution of buffered saline containing 0.2 per cent glucose the phosphocreatine reached a value of 30.5 mgm., i.e. 240 per cent increase over the original value.

Under the same conditions the muscle of 4 depancreatized dogs had an initial phosphocreatine content of 4.5 mgm. which increased to 21.8 mgm. after 2 hours in oxygen, i.e. an increase of 260 per cent. Simultaneous determinations of the respiratory quotient of the excised diabetic muscle averaged 0.73, i.e. practically complete diabetes.

In the presence of fat oxidation, therefore, a synthesis of phosphocreatine occurred which approaches, or even equals that taking place in the presence of carbohydrate oxidation.

Studies on Minute Hemolytic Streptococci. II. The Distribution of Minute Hemolytic Streptococci. By PERRIN H. LONG and (by invitation) ELEANOR A. BLISS, and CHARLES F. WOLCOTT, Baltimore, Md.

Recently we have described the occurrence of minute beta hemolytic streptococci in the rhinopharynges of individuals ill with diseases in which beta hemolytic streptococci are generally considered to be of etiological significance. In this report we propose to discuss the distribution of these minute or-

ganisms in normal individuals and in those ill with glomerular nephritis, scarlet fever, and progressive rheumatic infection and to consider their possible significance in certain phases of these diseases.

The Significance of the Serum Iron in Certain Types of Anemia. By J. F. McIntosh, Montreal, Canada.

Fontès and Thivolle, Warburg and Krebs, have shown that the serum iron is decreased in experimental anemias, in the horse, and in birds respectively.

These findings have been confirmed in the dog. After bleeding, the serum iron fell from 0.17 to 0.08 mgm. per cent, and remained low throughout the period of hemoglobin regeneration. After the latter had reached a normal figure, the serum iron returned to its initial value.

In cases of hypochromic anemia, the initial values may be low. During iron medication, the hemoglobin values tend to rise first, the serum iron later. Just as in experimental anemia, narrow fluctuations in the hemoglobin are accompanied by wide changes in the serum iron.

In an untreated case of pernicious anemia, a high initial value of 0.24 mgm. per cent was found. During the reticulocytosis produced by liver extract, the value fell to 0.04 mgm. It rose gradually as treatment was carried on.

The serum iron appears to represent a balance between hemoglobin synthesis, and the iron which is available from food and iron depots.

A Comparison of the Tuberculous and Chronic Non-tuberculous Pulmonary Infiltrations of Childhood as Regards Age-Incidence, Anatomical Distribution and Course. By F. M. McPhedran, Philadelphia, Pa.

In a pathogenetic study during the past eleven years serial x-ray observations have been made on three groups of families: (1) tuberculous, (2) normal controls, (3) families having a high incidence of chronic non-tuberculous pulmonary lesions. Both tuberculous and non-tuberculous infiltrations predominate in certain families, but after infancy the two lesions diverge widely in incidence and severity. From the third to the eleventh year severe tuberculous lesions are rare, while non-tuberculous bronchopneumonia, particularly chronic or relapsing bronchopneumonia causes severe illness. Towards puberty non-tuberculous lesions decrease both in extent and in the illness they cause. At the same time there begin to appear the dangerous tuberculous lesions of the adult type. During childhood the non-tuberculous lesions predominate in the lower third of the lung and are densest close to the cardiophrenic angle. The tuberculous lesions develop chiefly in the middle third and are based on the peripheral pleura. Excavation, if it occurs, is usually on the posterior wall. Non-tuberculous infiltrations appear not to intensify co-existing tuberculous lesions.

Having regard to the total of chronic non-tuberculous bronchopneumonias, bronchiectasis is relatively rare.

On a Pernicious Anemia-Like State in the Guinea Pig. By B. M. Jacobson (introduced by J. H. Means), Boston, Mass.

It has been found, in the investigation of several laboratory animals, that certain guinea pigs respond to the administration of therapeutically active commercial liver extracts with a reticulocytosis. Such guinea pigs are termed reactive animals; they maintain this state indefinitely. The minimum amount of material which evokes a positive reticulocyte response is the liver extract derived from approximately 0.6 mgm. of fresh porcine liver, per kilogram of

guinea pig. This is defined as the Guinea Pig Unit (G.P.U.) of hematopoietic activity. One hundred grams of fresh porcine liver may be said to contain approximately 164,000 G.P.U.

The pernicious anemia-like state in the reactive guinea pig is evidenced by the following phenomena. (1) Therapeutically potent commercial liver extracts show quantitatively similar activity in the guinea pig. On the other hand, numerous pure inorganic and organic substances, as well as crude mixtures obtained from biological materials, which are all inert in pernicious anemia, are likewise inert in the guinea pig. Further control data are furnished by the fact that although the liver of a non-anemic patient, when assayed on the guinea pig, showed an activity of 164,000 G.P.U. per 100 grams, the liver of a patient, who died of pernicious anemia, exhibited an activity of less than 12 G.P.U. per 100 grams. (2) The liver of the reactive guinea pig is deficient in hematopoietic activity, for such a liver has shown a potency of only 23,000 G.P.U. per 100 grams, while the liver of a non-reactive animal one of 164,000 G.P.U. per 100 grams. (3) Finally, the reactive guinea pig responds to the administration of a mixture of Castle's intrinsic factor (normal human gastric juice) and extrinsic factor, with a reticulocytosis; whereas inactivation of the intrinsic factor by heat results in the guinea pig, as in pernicous anemia, in a negative response.

It is therefore believed that the reticulocytosis induced in the guinea pig by commercial liver extracts is related to the same phenomena in pernicious anemia.

Further Observations on Chronic Idiopathic Hypochromic Anemia. The Effect upon Formation of Erythrocytes of Administering Small Doses of Inorganic Iron in an Acid Medium as Compared to Predigested Food with Special Reference to Beef Muscle. By STACY R. METTIER and (by invitation) FREDERICK KELLOGG, San Francisco, Cal.

Previous studies on patients with achlorhydria and chronic hypochromic anemia showed increased formation of blood following the daily administration of a meal that had been predigested in vitro with strong HCl and pepsin. The acidity was adjusted to pH 4 before feeding. The response was similar to that induced by large doses of inorganic iron. The meal (i.e., 300 grams of lean beef muscle, 200 grams of spinach and 2 soft boiled eggs) when ashed contained approximately 12 mgm. of iron. It was believed the effect induced was due to the organic iron of the food. Inorganic iron in such small daily doses is ineffective in this type of anemia. In the present study it was decided to determine whether or not the utilization of a very small dose of inorganic iron could be influenced by buffering it to an acid pH upon the addition of HCl, sodium citrate and a simple protein poor in iron. i.e., gelatin.

In addition it was decided to ascertain whether or not a predigested meal, consisting only of beef muscle meat, would influence blood formation favorably.

A series of patients were given daily, for 10 to 14 days, feedings of 13 or even 25 mgm. of inorganic iron with 50 grams of gelatin, the hydrogen-ion concentration of which was adjusted to pH 4 with the addition of HCl and 10 grams of sodium citrate. At the end of this time a second period was started when the predigested meal (see parenthesis above) was fed. No response in hematopoiesis was induced by the inorganic iron-gelatin combination, but the blood returned to within normal limits on the predigested meals.

In another series of experiments 200 grams of predigested beef muscle was fed daily during the first period and increased to 400 grams in the second period.

The smaller feedings of beef muscle failed to affect hematopoiesis but an increase in hemoglobin formation occurred after the larger feedings.

Apparently inorganic iron in small amounts in an acid medium with a simple protein has no effect on blood formation in contrast to a predigested meal containing a comparable amount of organic iron.

It is believed these studies give further support to the hypothesis that gastric dysfunction leads to the development of anemia in patients with chronic idiopathic hypochromic anemia.

Clinical Experiences with Thevetin, a Cardiac Glucoside. By HARRY ARNOLD, Honolulu, WILLIAM S. MIDDLETON, Madison, Wisconsin and K. K. CHEN, Indianapolis, Indiana.

The toxicity of be-still nuts, the fruit of *Thevetia neriifolia,* or the yellow oleander, has been recognized for many years. The digitalis-like action of thevetin has recently been thoroughly studied by Chen and Chen. This glucoside acts more promptly, but is less persistent in its cardiac effects than digitoxin. The cat unit for thevetin was established by Chen and Chen as 0.85 mgm. per kilogram or about one-seventh of the potency of ouabain.

Clinical trial of thevetin has been pursued with results justifying continuance. In all instances the theoretical digitalis tolerance has constituted a guide for dosage and the level of five cat units at a single intravenous dose and ten cat units in the twenty-four hours has not been exceeded. The subjective and objective evidences of decompensation have been regularly controlled, as a rule before the appearance of the earlier gastrointestinal manifestations of toxemia, such as anorexia and nausea. The pulse has been slowed more in patients with auricular fibrillation than in those with normal rhythm. A single subject suffering from auricular paroxysmal tachycardia showed no slowing of the rate under thevetin. Electrocardiographic changes parallel those of ouabain.

Experimental Study of Clinical Vitamin B Deficiency. By KATHARINE O'SHEA ELSOM (introduced by T. Grier Miller), Philadelphia, Pa.

It is commonly believed that mild vitamin B deficiency occurs only very rarely in man and that for such deficiency to develop at all, the diet must be grossly restricted, as it is in beriberi, or that gross gastro-intestinal disease must exist which interferes with the absorption of food. Observation of patients encountered in the Gastro-Intestinal Section of the University of Pennsylvania Hospital made it seem possible that this belief might be erroneous. Accordingly, a clinical experiment was devised to determine the effects upon man of a moderate restriction of the vitamin B complex, to determine the clinical signs of mild vitamin B deficiency and to ascertain the relative importance of various fractions of the B complex in relief of such deficiency.

This paper reports the results obtained when a patient whose symptoms were suspected of being due to habitually inadequate diet was placed for one year on a diet from which the major vitamin B containing foods were removed but which was adequate in every other known dietary requirement. Protein, caloric and fluid intakes were kept constant, the patient being under careful supervision in the hospital throughout the entire time of observation. After 5 months on the experimental diet various fractions of the vitamin B complex were added in series. Separate preparations were made of vitamins B_1 and B_2. A powdered brewer's yeast concentrate was used as a source of additional B factors. Prominent symptoms which became exaggerated during the five months when the patient received the experimental diet without added vitamin

fractions were: fatigue, anorexia, epigastric distress, constipation, sore tongue, pains and parethesias in the extremities. The most striking physical signs were: loss of weight, development of smooth tongue and of pitting edema of the extremities, the loss of vibratory sensation in the lower extremities and exaggeration of reflexes. Detailed studies were made of the weight loss, the edema, the alterations in total serum protein, the slight anemia and the evidences of impaired cardiovascular and gastro-intestinal functions which developed during the deficient period. Some improvement was observed to take place when separate preparations of vitamins B_1 and B_2 were added to the experimental diet. The whole yeast concentrate, however, appeared to be more effective in permanent relief from the symptoms, the physical signs and the evidences of disturbed function which had become manifest during the deficient period.

There are also briefly described similar results which were obtained upon another patient who had for ten years experienced symptoms and physical signs analogous to those which developed in the experimental patient.

Bile Pigment and Hemoglobin Regeneration. The Effect of Bile Pigment in Cases of Chronic Hypochromic Anemia. By ARTHUR J. PATEK, JR. (by invitation) and GEORGE R. MINOT, Boston, Mass.

Nine selected patients with chronic hypochromic anemia were studied to determine whether bile pigment could assist in hemoglobin production.

Concentrated bile pigment alone caused not a reticulocyte response but an increase of hemoglobin, about 7 per cent in 10 days. This indicates that in certain anemic patients pigment can be absorbed from the gastro-intestinal tract for building hemoglobin. No further increase of hemoglobin occurred when pigment feeding was continued for longer than about ten days.

After a reticulocyte response occurred to a suboptimal dose of iron, bile pigment was fed directly with the same dose of iron, and there followed a second reticulocyte response. The second response was sometimes of greater magnitude than the first. This indicates that bile pigment in some unknown manner can facilitate either iron absorption or utilization.

One patient, who could not obtain a normal hemoglobin level with large doses of iron, promptly increased her hemoglobin concentration when bile pigment was fed in addition to iron.

It is suggested that in certain cases of hypochromic anemia in addition to iron deficiency there may be a deficiency of a useful material, that is contained in bile pigment.

The Urinary Excretion of Cholesterol and Protein in Bright's Disease. By MAURICE BRUGER (introduced by H. O. Mosenthal), New York, N. Y.

The paucity of detailed studies in the past of the cholesterol excretion in the urine in Bright's disease and especially of the relation of the cholesterol to the protein excretion prompted this investigation. The cholesterol content of the urine was determined by methods developed in this laboratory in 23 cases of Bright's disease (chronic diffuse glomerular nephritis, 9 cases; chronic diffuse glomerular nephritis with a nephrotic component, 4 cases; amyloid nephrosis, 5 cases; lipoid nephrosis, 5 cases). The protein partition was investigated in all urine specimens and, in many instances, the cholesterol and protein contents of the urinary sediment were also determined. In 9 cases of Bright's disease, the diurnal variations in the urinary excretion of protein and cholesterol were studied. The results indicate that (a) the excretion of choles-

terol in the urine varies directly with the protein excretion; (*b*) the cholesterol content of the blood apparently exerts little influence on the concentration of cholesterol in the urine; (*c*) an increased excretion of globulin in the urine is accompanied by an augmented excretion of cholesterol esters, whereas the urinary excretion of a proportionately greater amount of albumin is generally accompanied by a larger output of free cholesterol; (*d*) the urinary sediment in Bright's disease is relatively low in protein but the cholesterol content may amount to as much as 30 per cent of the total urinary cholesterol; (*e*) the concentration of cholesterol and protein in the urine is usually diminished during the sleeping hours, but because of the frequent occurrence of nocturnal polyuria, the total output of these constituents is often greater during this period than in the waking hours.

The Estimation of Changes in Body Fluids. By PAUL H. LAVIETES (introduced by John P. Peters), New Haven, Conn.

The calculation of water exchange by the method proposed by Newburgh and Johnston and simplified by Peters, Kydd and Lavietes fails to give reasonable results in our hands, probably because in our patients insensible perspiration fails to parallel metabolism and because a practical error of considerable magnitude is incurred in the measurement of dry weight of ingesta and excreta or in the estimation of dietary foodstuffs, or in both. Furthermore at best this method does not distinguish between water exchange in the interstitial fluids and in the cellular fluids.

An independent method of estimating total water exchange from electrolyte metabolism has been devised on the assumption that the concentrations of sodium plus potassium in serum water is approximately equal to and varies with that in body water as a whole. Further, by assuming that practically all the sodium and chloride in the body are restricted to the interstitial fluids and that the concentrations of these electrolytes in these fluids are approximately identical with the concentrations in serum water, changes in interstitial fluid volume may be estimated from the metabolism of either sodium or chloride.

Normal and diseased subjects have been studied during periods of large exchange of water induced by various means. The estimation of total water exchange from sodium plus potassium metabolism in many instances gives more reasonable values than does that derived from Newburgh's method. Furthermore, interstitial fluid exchange calculated independently from sodium and chloride changes shows remarkable agreement in most cases.

The Development of Hypersensitiveness in Man. By FRANK A. SIMON (by invitation) and FRANCIS M. RACKEMANN, Boston, Mass.

According to the observations of Hooker, of Park, of Tuft, and more recently of T. Duckett Jones, sensitization as determined by a positive skin test can be produced artificially with ease and regularity when diphtheria antitoxin or rabbit peritoneal fluid is injected intracutaneously.

Our own experiments, made with guinea pig serum, confirm the findings of Jones in every way. Furthermore, normal and allergic individuals give the same results. Reactions of the delayed tuberculin type develop after the second or third intracutaneous treatment, whereas reactions of the immediate urticarial type are observed later, after the fifth to seventh treatment. As Jones says, these two reactions represent different phases of the immune process. When the doses were given always into the same spot, the degree of sensitiveness was

not greater in the reinjected area than elsewhere except in one case. Antibodies in the blood were demonstrated only when the skin reactions became large.

In other experiments, local sensitiveness of the nasal mucosa was produced by repeated packs wet with guinea pig serum. The character and time relation of the nasal response was comparable to the skin response.

Finally, and in marked contrast to our positive results with guinea pig serum, all attempts to produce a positive skin reaction to egg white by repeated intra-cutaneous doses, have so far failed. Presumably this failure depends upon the diet.

The Parenteral Administration of Sodium Ferrocyanide in Anemia. By JAMES M. BETHEA (by invitation) and PAUL REZNIKOFF, New York, N. Y.

Oral administration of iron in iron deficient anemia may be unsatisfactory because of inadequate absorption, intolerance or, perhaps, the necessity for rapid response. Such conditions, although uncommon, make parenteral administration desirable.

Organic iron, although non-toxic, is expensive. Inorganic preparations in which iron is in the cation are locally toxic, precipitating proteins. We have used a ten per cent solution of sodium ferrocyanide in which iron is in the anion. This does not precipitate proteins in vitro and is non-toxic locally or generally when given parenterally. The icteric index in patients during administration showed no change and daily urinalyses showed albumin and red blood cells in only a small percentage of patients. The drug was administered parenterally (25 to 30 mgm. of iron daily) to 12 patients with iron deficient anemia and to 13 rabbits (10 to 20 mgm. of iron daily) with anemia caused by bleeding or by a milk diet. In the patients some reticulocyte response and subsequent rise in hemoglobin in uncomplicated cases occurred. In anemic rabbits marked response occurred. The ferrocyanide is, however, excreted up to 90 per cent in the urine of patients. In rabbits much less is excreted if the animal is markedly anemic.

The Effects of Repeated Injections of Histamine on Gastric Secretion. By LEON SCHIFF, Cincinnati, Ohio.

Over 400 subcutaneous injections of histamine have been given to one individual during a period of over two years. Continuous aspirations of the gastric juice have been made under the same conditions on over 200 occasions for periods of $2\frac{1}{2}$ hours. An increase in the volume of gastric secretion with no increase in the total output of acid has been observed following single injections of histamine. A permanent increase in the volume of gastric juice obtained under fasting conditions without histamine has also been noted. In following the patient's general condition, with particular reference to the gastrointestinal tract and hematopoietic system, no harmful effects could be detected. The effects of atropine and pilocarpine have also been studied.

The Etiology of Whooping Cough. By GERALD S. SHIBLEY, Cleveland, Ohio.

The Bordet-Gengou bacillus is considered by many to be the specific causative agent of whooping cough. Others believe that there is a virus component. In an effort to settle this question, freshly isolated *H. pertussis* was maintained in the so-called infective phase (" S ") for 8 months; then, being theoretically virus free, was used to inoculate a chimpanzee. Whooping cough appeared after a 10 day incubation period. It was typical clinically, hematologically, bacteriologically, and at postmortem, pathologically (except for absence of

organisms in ciliary apparatus). The laboratory phase, (" R "), presumably avirulent had failed previously to produce the disease in another chimpanzee. In spite of rigorous precautions two other chimpanzees in the adjoining room contracted coughs which lasted 6 weeks and were very suggestive of pertussis. The animals were not tame enough for successful cultural study. Subsequent attempts to produce whooping cough in these animals by the method described above failed, as would be expected if they had had the disease. The presence of immunity in the animals has been confirmed by their failure to contract the disease when inoculated with *H. pertussis* contained in pooled sputa from human cases.

Physiological Relationships of Common Clinical Data in the Diagnosis of Myocardial Disease. By ISAAC STARR, JR., Philadelphia, Pa.

The application of cardiac output methods to patients requires so much apparatus and technique that there is little likelihood that they will become an aid in routine diagnosis. However, increased knowledge of cardiac physiology in disease, gained by means of cardiac output methods, may be expected to aid the diagnosis of cardiac disease by permitting more correct interpretation of the ordinary clinical data.

As a result of estimations of cardiac output on 250 patients (Starr, Donal, Margolies, Shaw, Collins and Gamble (in press)), relationships have been demonstrated to exist between cardiac work and heart volume, cardiac output and metabolism, cardiac output and body weight, etc. These relationships have been expressed mathematically and the resulting formulae combined so that the factor " cardiac output " is eliminated. As a result we have obtained a number of formulae which express normal physiological relationships and may be used for the detection of abnormal myocardial function without the necessity of estimating cardiac output. These formulae have been applied to the data of normal persons, and the normal limits determined. The great majority of patients with undoubted myocardial disease give results far outside the normal limits.

Examples of such formulae, expressed in the ordinary clinical units, follow.

$$(1) \quad \frac{\text{Mean blood pressure}}{\text{Pulse rate}} \; (78 + 1.2 \text{ oxygen consumption}) - 0.35 \; (\text{heart silhouette area})^{3/4} = K,$$

$$(2) \quad \frac{\text{Mean blood pressure}}{\text{Pulse rate}} \; (45 + 0.9 \text{ weight kilos}) - 0.35 \; (\text{heart silhouette area})^{3/4} = K.$$

The lower normal limits are approximately $K = -50$ and $K = -100$ respectively; (2) may not be used in hyperthyroidism or hypertension. Neither gives evidence of coronary disease.

Relationships Derived from Measuring Solutes in Blood or Serum on a Water Basis. By F. WILLIAM SUNDERMAN and (by invitation) ENNION WILLIAMS, Philadelphia, Pa.

With respect to solutions containing a high concentration of solids, as for example blood or serum, the distinction between concentration of solute per unit amount of solution and per unit amount of solvent becomes important. The concentration of solute per volume of solution is the relationship usually employed in clinical studies. It is, however, the concentration of solute per unit amount of solvent which has especial physicochemical significance and is related to osmotic equilibrium and to the activity of the solute.

Relationships of significance which we have derived from the measurement of solutes of serum and cells on a water basis are the following:

(1) Following the ingestion of glucose by diabetic patients, the increase in the molal concentration of glucose is shown to be largely compensated for osmotically by the decrease in the molal concentration of chloride in the serum.

(2) By the measurement of solutes in serum on both a water and volume basis, it can be shown that the rise of glucose in the serum of diabetic patients tends to draw not only water but saline from the tissues. The water added to the serum dilutes the serum electrolytes and causes an inflow of total base and chloride from the tissues.

(3) We confirm MacKay's demonstration that there is equal distribution of sugar between the water of the corpuscles and the water of the serum although not an equal distribution between the serum and corpuscles on a volume basis.

(4) A linear correlation is demonstrated between the specific gravity and the water content of serum. This permits us to employ the relatively simple and dependable measurement of the specific gravity of serum without the additional measurement of its dry weight in order to calculate the concentration of a solute per unit amount of water in the serum with accuracy sufficient for many purposes.

The Flow of Lymph as a Measure of Filtration from the Capillaries in Normal and Edematous Dogs. By A. A. WEECH and (by invitation), E. GOETTSCH, New York, N. Y.

The study deals with a comparison of the relative rates of the filtration of fluid across the capillaries in normal dogs and in dogs exhibiting edema due to depleted plasma proteins. Data concerning capillary filtration were obtained by observing rates of lymph flow. When a cannula is first introduced into one of the main lymphatic trunks of the ankle and the flow of lymph stimulated by allowing the animal to walk about, the rate of flow is at first rapid but as collection is continued it becomes slower and finally remains relatively constant over long periods of time. The rapid initial rate probably results from the withdrawal of fluid which had previously accumulated in the interstitial spaces. The subsequent constant rates must correspond to the production of fresh lymph, that is, to the rate of filtration from the capillaries. Inasmuch as the number and diameter of lymphatic vessels in the region of the ankle is not subject to wide anatomical variation,[1] the average lymph flow in a group of animals should be roughly proportional to capillary filtration in the region distal to the cannula, i.e., the foot. Among 13 normal dogs, studied in this manner, the constant rates of lymph flow varied between 0.05 cc. and 1.96 cc. per half hour and averaged 1.13 cc. per half hour. Among 8 edematous dogs the analogous rates varied from 0.01 cc. to 4.10 cc. per half hour and averaged 1.24 cc. per half hour. In one case only was the rate of flow greater than that included in the range for normal animals. In the normal group analyses of serum revealed an average total protein concentration of 5.70 grams per cent, of which 3.33 grams were albumin and 2.37 grams were globulin. The average colloid osmotic pressure of the serum calculated by the formula of Wells, Youmans, and Miller[2] was 23.5 cm. of water. In the edematous group the average serum

[1] According to White, Field, and Drinker (Am. J. Physiol., 1933, 103, 34) there are "three or at the very most four" lymphatic vessels of approximately equal size in this region.

[2] Wells, H. S., Youmans, J. B., and Miller, D. G., Jr., J. Clin. Invest., 1933, 12, 1103.

viously been reported by Yater. These two cases constituted the only cases ever studied in this way. Only six cases of congenital heart block including the present one, have been studied by necropsy. In all six, congenital defects in the septum between the right and left sides of the heart were present. The present case is that of a child who lived 18 hours, during which time he was intensely cyanotic. Numerous electrocardiograms were taken, all showing complete heart block, and the effect of atropine was studied. An x-ray film of the chest was also taken. Examination of the heart showed practically complete absence of the interauricular septum and of most of the membranous portion of the interventricular septum. Serial sections showed that there was practically complete separation of the auricles and ventricles by the central fibrous body in the region where the specialized muscle bundle normally bridges the auriculoventricular fibrous junction. The bundle of His was well formed but was disconnected from the auriculoventricular node in this region. Lantern slides are shown of the electrocardiograms, roentgenogram, macroscopic appearance of the heart and of illustrative microscopic sections.

The Relationship of the Intrinsic Factor to a Hematopoietic Material in Concentrated Human Gastric Juice. By O. M. HELMER and PAUL J. FOUTS (by invitation), and L. G. ZERFAS, Indianapolis, Indiana.

The demonstration of a substance in normal human gastric juice capable of inducing a reticulocytosis when injected intramuscularly into patients with Addisonian pernicious anemia during blood relapse, has been found to be dependent upon the process of concentration of the gastric juice by vacuum distillation and upon the presence of both the intrinsic factor and an extrinsic factor or factors. As the intrinsic factor was destroyed or altered during the process of formation of the hematopoietic substance, the substance formed is not identical with the intrinsic factor.

THE EFFECT OF CORONARY OCCLUSION UPON THE INITIAL PHASE OF THE VENTRICULAR COMPLEX IN PRECORDIAL LEADS

By SAMUEL BELLET and CHARLES G. JOHNSTON

(*From the Robinette Foundation, the Department of Research Surgery. University of Pennsylvania, and the Division of Cardiology, Philadelphia General Hospital, Philadelphia*)

In electrocardiograms of normal human hearts recorded by precordial leads the initial ventricular complex shows a fairly constant configuration. With the right arm electrode placed at the apical region and the second electrode at any point distant from the heart the primary ventricular deflection consists of a conspicuous downward deflection followed by an upward deflection. The initial downward deflection which is constantly present in normal controls entirely disappears or becomes conspicuously smaller after infarction involving the apex, lower anterior and lateral portions of the left ventricle [1] (8) (10) (11). Moreover, the initial downward deflection is influenced by the position of the anterior electrode as well as by pathological changes within the heart, decreasing in amplitude and even disappearing as the electrode moves from the apex toward the base of the heart.

The electrocardiogram of the dog and the cat, obtained by precordial leads is similar to that observed in the human. Moreover, the influence of change of position of the anterior electrode resembles that occurring in man. Because of this similarity and because the use of animals allows the changes to be studied at frequent intervals from the very moment of muscle injury until repair and recovery have reached their maximum, we have used these animals to study the effects of myocardial infarction upon the initial downward deflection of precordial leads.

In general, the experiments fall into two groups (1) acute and (2) survival experiments. In the former, the effect of muscle injury produced by ligation of different coronary arteries, or the damage resulting from the application of a cautery directly to the heart muscle upon the initial downward deflection was observed over a period of about two hours. In the survival experiments, coronary arteries were ligated and the electrocardiographic change followed over a period of months.

The purpose of this paper is to record the results of experiments in dogs

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[1] Occasionally this deflection disappears when apical involvement alone is present.

and cats designed to show the location and extent of myocardial lesions necessary either to modify or cause the disappearance of the initial downward deflection of precordial leads.

METHODS

The animals were anesthetized by the administration intraperitoneally, of 50 mgm. of sodium amytal per kilogram of body weight. Artificial respiration was instituted by means of a tracheal cannula and a bellows. The electrodes were attached for taking indirect leads; additional electrodes were sutured beneath the skin of the anterior and posterior cardiac region so that precordial leads could also be obtained. Three precordial leads were routinely taken in our experiments, anteroposterior (Lead IV), anterior to left leg (Lead V), and posterior to left leg (Lead VI). The right arm wire was attached to the anterior, and the left arm wire to the posterior, an arrangement which in the normal animal yielded an electrocardiogram having an initial downward deflection followed by an upward deflection.

The chest was opened by cutting the second, third, fourth and fifth ribs along the left posterior axillary line; the heart was exposed by opening the pericardium and the selected coronary arteries were ligated. The heart was then returned to its original position, the ribs and skin were approximated and electrocardiograms taken at frequent intervals. In some animals the pericardium was closed, in others it was not sutured; there was no difference in the resulting electrocardiograms. Control studies showed that merely opening and closing the chest in the manner described was without significant effect upon the electrocardiograms. In the acute experiments where the myocardium was injured by cauterization of selected regions of the heart instead of coronary artery ligation, this injury was accomplished by an electric cautery at red heat applied until the superficial portion of the myocardium was practically carbonized. At the end of both types of experiment, which usually lasted not more than two hours, the animals were sacrificed.

RESULTS

The effects of the procedures just suggested upon the initial downward deflection of precordial leads were to some extent complicated; for clarity the result of each procedure will be presented separately.

Ligation of branches of the coronary arteries

Ligation of the anterior descending branch of the left coronary artery, performed in 12 dogs and in 6 cats, produced consistent R–T interval deviations of the type described by Wood and Wolferth (14) but failed to alter the initial downward deflection.

Dogs:

Ligation in three dogs of the septal branch of the left coronary artery produced no change in the initial downward deflection.

Ligation in five dogs of the anterior divisions of the circumflex branch of the left coronary artery was without influence upon the initial downward deflection.

Ligation of the anterior descending plus the septal branch of the left coronary artery resulted in a slight diminution of the initial downward deflection in some experiments. In those experiments, where the anterior branches of the circumflex were ligated in addition to the two vessels of the previous experiments, the animals died soon afterward, usually within a few minutes after the last vessel was tied. Electrocardiograms taken during this brief period showed no change in the initial downward deflection.

Cats:

Ligation of single coronary arteries in dogs and the combinations above mentioned having failed to cause a disappearance of the initial downward deflection, ligation of similar vessels was next attempted in the cat. That cats can withstand multiple coronary artery ligations much better than dogs has been demonstrated to our satisfaction by numerous experiments. The heart of the cat can beat vigorously for long periods after ligation of one and often after ligation of the two main branches of the left coronary artery.

Ligation of the anterior descending branch in cats resulted in R–T deviations, but without disappearance of the initial downward deflection in Lead IV of the precordial leads. Ligation in four cats of the anterior descending branch of the left coronary artery plus the anterior branch of the circumflex resulted in a complete disappearance of the initial downward deflection and an R–T interval deviation situated below the iso-electric line, the resulting electrocardiogram (Fig. 1) being practically identical with that observed in the human after acute occlusion of the anterior descending branch of the left coronary artery (11) (13).

A   B   C
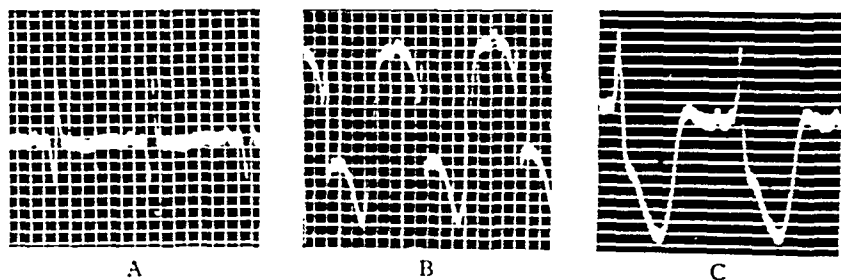
FIG. 1.[1] SHOWING THE EFFECT IN LEAD IV OF LIGATION OF THE ANTERIOR DESCENDING BRANCH OF THE LEFT CORONARY ARTERY PLUS THE ANTERIOR BRANCH OF THE CIRCUMFLEX ARTERY IN THE CAT

(*A*) Normal. (*B*) After ligation of the anterior descending branch of the left coronary artery. (*C*) After ligation of the anterior descending and circumflex branches of the left coronary artery: note the absence of the initial downward deflection and S–T interval situated below the iso-electric line.

[1] The initial phase of the ventricular complex, which shows clearly in the negatives, has been retouched for purposes of reproduction in this and subsequent figures.

Cauterization of the heart muscle

Having observed the results of ligation of large coronary arteries, an endeavor was made to compare the results of cauterization of the heart muscle; first, because the area of damage brought about by this method may be accurately controlled, and secondly, because a slightly different type of muscle injury is produced.
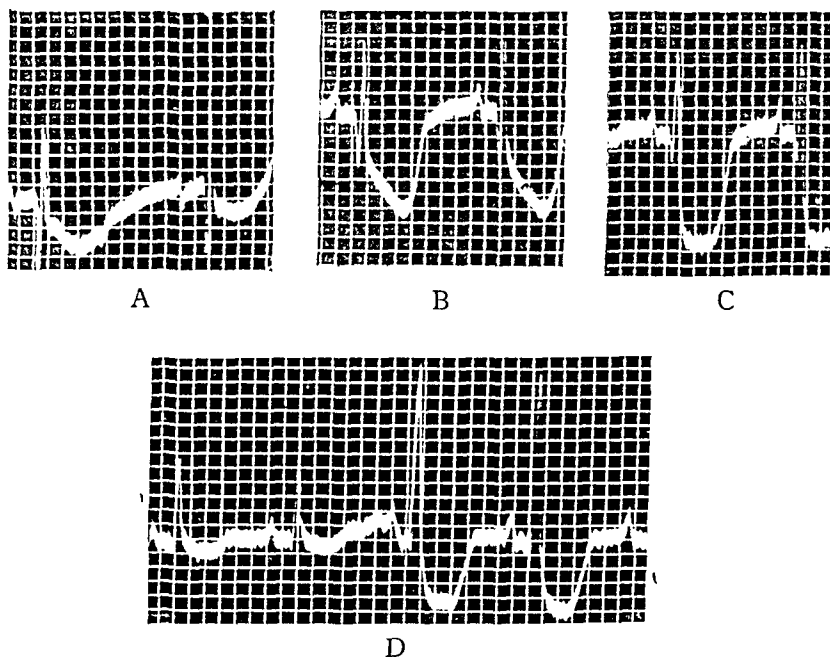
FIG. 2 (Experiment August 25, 1932). SHOWING THE EFFECTS IN LEAD IV OF CAUTERIZATION OF THE HEART MUSCLE IN DOGS

(*A*), (*B*), (*C*), (*D*) show the effect of cauterization of the heart muscle. (*A*) only small portion of apex cauterized, note the depression of S–T interval; the initial downward deflection is little affected. (*B*) Area of cauterization increased to involve lower third of anterior wall of the left ventricle; note the increase in depression of the S–T interval. The initial downward deflection is slightly diminished in amplitude. (*C*) Area of cauterization increased to involve lower two-thirds of the anterior wall of left ventricle including the apex and a portion of right ventricle adjacent to interventricular groove. Note the almost complete absence of initial downward deflection and marked depression of the S–T interval. (*D*) Same as *C*. Note the variation in the ventricular complex with respiration, the largest complexes occur with inspiration, the smallest with expiration. Note the depression of S–T interval and the absence of the initial downward deflection.

In four experiments in dogs in which the apex, and the lower half of the anterior and lateral walls of the left ventricle were cauterized, the initial downward deflection disappeared in only one dog; in the remaining three the initial downward deflection, while diminished, did not disappear. In three other experiments the entire anterior portion of the heart (both right

and left ventricles) and the lateral portion of the left ventricle were cauterized before the initial or downward deflection disappeared (Fig. 2). In three other dogs where a similar portion of the heart was cauterized the initial downward deflection, while diminished did not disappear. Apparently the extent of the cauterized area is an important factor in determining the final results.

In cats no conspicuous change in the initial downward deflection was observed when the right ventricle alone was cauterized. The amplitude of this deflection was diminished most when the apex, and the lower anterior and lateral portions of the left ventricle were cauterized.

In three cats the initial downward deflection disappeared and an R–T deviation situated below the iso-electric line resulted after cauterization of the apex and the lower two-thirds of the anterior and lateral walls of the left ventricle, including a narrow strip of right ventricle adjacent to the anterior interventricular groove.

The ventricular complexes in these experiments as well as the others in which the chest had been opened, often showed a phasic variation with respiration, the highest amplitude being attained at inspiration, the lowest at expiration (Fig. 2D). The disappearance of the initial downward deflection was always judged from the complexes with the highest amplitude.

Effect of injury of a small area of heart muscle upon the initial downward deflection of the electrogram

The purpose of this experiment was to compare the electrogram from an electrode placed directly on a small injured portion of the heart muscle with the tracings obtained from the precordial leads after such injury.

The heart was exposed and the animal arranged for taking electrograms as well as precordial leads. In leading off from the heart directly, electrodes soaked in copper sulphate contained in narrow glass tubes were used. An area about a centimeter and a half in diameter on the anterior surface of the left ventricle near the apex was selected and control electrograms taken. The right arm electrode was the one which led off from the heart directly, the left arm electrode being in contact with the chest posteriorly; the left leg electrode was sutured beneath the skin of the left leg as in previous experiments. That portion of the left ventricle in contact with the direct lead electrode was then cauterized and the electrogram repeated. A control was again taken from an uninjured portion of the heart on the anterior surface of the right ventricle. Needle electrodes were then inserted into the myocardium immediately beneath the cauterized area and electrograms again taken.

In the control tracings with the electrode placed directly upon the normal muscle of the left ventricle the configuration of the electrogram was similar to that of the electrocardiogram of the precordial lead; an initial downward deflection was followed by an upward deflection, the relative size

FIG. 3. SHOWING THE EFFECT OF CAUTERIZATION OF PORTIONS OF THE MYOCARDIUM UPON THE ELECTROGRAM DIRECTLY FROM THE INJURED AREA AND THE ELECTROCARDIOGRAM OF PRECORDIAL LEAD IV

(*A*) Precordial Lead IV; note the presence of large initial downward deflection. (*B*) Electrogram taken from portion of left ventricle near apex; note presence of large initial downward deflection. (*C*) Electrogram from this same area after this portion of the heart muscle had been damaged by cauterization. Note that the initial downward deflection is now entirely absent. (*D*) Electrogram from the uninjured portion of the right ventricle; note the maintenance of the initial downward deflection. (*E*) Needle electrode inserted into the injured area of left ventricle. (*F*) Precordial Lead IV after cauterization had been extended to include the apex and lower third to half of the left ventricle (the right ventricle was uninjured). Note the absence of initial downward deflection.

of which varied. depending upon the location of the anterior electrode. When the electrode was placed on the anterior surface of the left ventricle near the apex, the initial downward deflection was approximately equal to that of the upward deflection.[2]

Cauterization of this small area near the apex of the left ventricle resulted in a disappearance of the initial downward deflection in the electrogram taken from the electrode placed on the cauterized area. whereas a control electrogram taken immediately afterwards from an uninjured portion of the heart on the anterior surface of the right ventricle showed the initial downward deflection still preserved (Fig. 3). Needle electrodes inserted deep into the muscle below the cauterized area showed an absence of the initial downward deflection.

Infarction of the posterior wall

Ligation of the coronary arteries supplying the posterior wall or injury of this part of the myocardium by cauterization produced no change in the initial downward deflection. The only change observed was that the R–T interval was situated above the iso-electric line. originating from the downstroke before it reached the base line (Fig. 4). These findings are similar to those observed in man (11) (13).

IV   V   VI   IV
            A   B

FIG. 4.   SHOWING THE EFFECT OF INJURY TO THE HEART MUSCLE ON THE
POSTERIOR WALL OF THE LEFT VENTRICLE IN CAT

(A) Precordial Leads IV. V. and VI before injury. Note the presence of initial downward deflection in Leads IV and V equal in amplitude to the upward deflection.   (B) Shows the effect in Lead IV of cauterization of the posterior wall of the left ventricle. Note that in B there is no change in the initial downward deflection; the only alteration observed being that the S–T interval comes off considerably above the isoelectric line and has a monophasic character.

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[2] In the electrogram with one electrode on the left ventricular surface and the other at some distant point the deflection obtained was approximately 20 times the value obtained in the indirect leads (5) (7). It was. therefore. necessary to tighten the string about 20 times the normal to obtain tracings with an amplitude approximating that usually seen in the indirect leads.   In taking the electrogram from the surface of the right ventricle the string had to be tightened less than when the electrode was placed on the left ventricle. probably indicating that the voltage developed by the right ventricle is less than that developed by the left (4).

FIG. 3.   SHOWING THE EFFECT OF CAUTERIZATION OF PORTIONS OF THE MYOCARDIUM UPON THE ELECTROGRAM
DIRECTLY FROM THE INJURED AREA AND THE ELECTROCARDIOGRAM OF PRECORDIAL LEAD IV

(A) Precordial Lead IV; note the presence of large initial downward deflection. (B) Electrogram taken
from portion of left ventricle near apex; note presence of large initial downward deflection. (C) Electrogram
from this same area after this portion of the heart muscle had been damaged by cauterization. Note that the
initial downward deflection is now entirely absent. (D) Electrogram from the uninjured portion of the right
ventricle; note the maintenance of the initial downward deflection. (E) Needle electrode inserted into the in-
jured area of left ventricle. (F) Precordial Lead IV after cauterization had been extended to include the apex
and lower third to half of the left ventricle (the right ventricle was uninjured). Note the absence of initial
downward deflection.

of which varied, depending upon the location of the anterior electrode. When the electrode was placed on the anterior surface of the left ventricle near the apex, the initial downward deflection was approximately equal to that of the upward deflection.[2]

Cauterization of this small area near the apex of the left ventricle resulted in a disappearance of the initial downward deflection in the electrogram taken from the electrode placed on the cauterized area, whereas a control electrogram taken immediately afterwards from an uninjured portion of the heart on the anterior surface of the right ventricle showed the initial downward deflection still preserved (Fig. 3). Needle electrodes inserted deep into the muscle below the cauterized area showed an absence of the initial downward deflection.

Infarction of the posterior wall

Ligation of the coronary arteries supplying the posterior wall or injury of this part of the myocardium by cauterization produced no change in the initial downward deflection. The only change observed was that the R–T interval was situated above the iso-electric line, originating from the downstroke before it reached the base line (Fig. 4). These findings are similar to those observed in man (11) (13).

IV   V   VI   IV
              A   B

FIG. 4. SHOWING THE EFFECT OF INJURY TO THE HEART MUSCLE ON THE POSTERIOR WALL OF THE LEFT VENTRICLE IN CAT

(A) Precordial Leads IV, V, and VI before injury. Note the presence of initial downward deflection in Leads IV and V equal in amplitude to the upward deflection. (B) Shows the effect in Lead IV of cauterization of the posterior wall of the left ventricle. Note that in B there is no change in the initial downward deflection; the only alteration observed being that the S–T interval comes off considerably above the isoelectric line and has a monophasic character.

[2] In the electrogram with one electrode on the left ventricular surface and the other at some distant point the deflection obtained was approximately 20 times the value obtained in the indirect leads (5) (7). It was, therefore, necessary to tighten the string about 20 times the normal to obtain tracings with an amplitude approximating that usually seen in the indirect leads. In taking the electrogram from the surface of the right ventricle the string had to be tightened less than when the electrode was placed on the left ventricle, probably indicating that the voltage developed by the right ventricle is less than that developed by the left (4).

SURVIVAL EXPERIMENTS

METHODS

In the chronic type of experiment the animals were operated upon under sodium amytal anesthesia with strict asepsis. A cannula was introduced through the mouth into the trachea and pulmonary inflation was maintained by means of a bellows. The chest was opened by an incision in the fourth interspace on the left side. By means of a self retaining retractor the fourth and fifth ribs were separated. The pericardium was longitudinally incised and drawn into the wound in the chest wall, thus preventing the expanding and contracting lungs from interfering during the exposure and ligation of the coronary artery. The artery of choice was dissected free from the epicardium and doubly ligated with silk ligatures. In all the survival experiments the anterior descending branch of the left coronary artery was the vessel ligated. The pericardium was then closed by interrupted sutures and the wound in the chest closed with the lungs in full expansion. Following the closure of the chest wall the tracheal cannula was removed. The animals were kept on the usual animal-house diet and allowed a minimum amount of exercise. Although the mortality was high we obtained five dogs which were followed for from 3 to 6 months after operation. Electrocardiograms were taken before operation, at frequent intervals after the ligation of the coronary artery during the first 24 hours, daily for the next 10 days, and weekly during the chronic stage.

In most instances where death occurred, it took place either immediately or soon after the ligation of the coronary artery. If the animal survived 24 hours the chances for its recovery were good. The animals that recovered appeared to be in good condition and about a week after operation acted like normal animals. One dog, Number 324, died three months after operation. The remaining four dogs were sacrificed four to six months after operation. We record also the results on one cat which died from pneumonia 36 hours after operation.

Electrocardiograms and pathologic findings

Dog Number 261 (Fig. 5). The initial downward deflection originally prominent, had considerably diminished 7 hours after operation and was minimal from the 3d to the 7th day after operation. It tended thereafter to return gradually toward normal but never attained its preoperative amplitude. Forty-five days after operation the initial downward deflection was about one-half that of the upward deflection. The R–T interval changes resembled those observed in the human subject. The high amplitude of the (upright) T waves in Leads IV and V were practically identical with those observed in man and probably represent a sub-acute stage of infarction (13) (15). T waves of this type were observed only from the 3d to the 5th day after ligation of the coronary artery. After about the 9th day the electrocardiogram remained practically constant, and appeared to be that characteristic for the chronic stage.

This animal when sacrificed six months after operation showed dense pericardial adhesions on the anterior surface of the heart around the interventricular groove. An area of infarction about two centimeters in diameter involved chiefly the apex of the left ventricle, with beginning aneu-

rysmal dilatation. The area of fibrosis was more marked on the endo-
cardial than on the epicardial side of the left ventricle (Fig. 6).

Dog Number 297. The initial downward deflection was diminished
somewhat four hours after ligation and was absent after 24 hours. It
remained absent for 5 days and then gradually reappeared, but never re-
turned to its preoperative value, and never exceeded about half the value
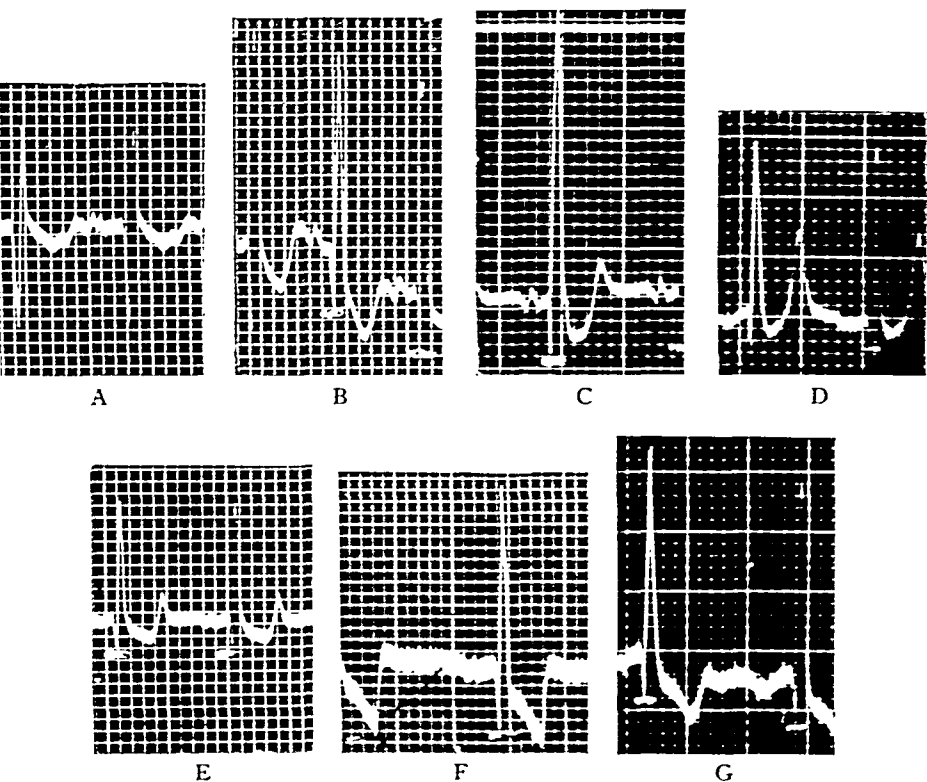
FIG. 5. SHOWS THE ELECTROCARDIOGRAMS FROM PRECORDIAL LEAD IV
FROM THE SURVIVAL EXPERIMENT ON DOG NUMBER 261

(*A*) before operation. (*B*) Seven hours after ligation of the anterior de-
scending branch of left coronary artery. Note the marked diminution in the
initial downward deflection and the slight depression of S–T interval. (*C*) 44
hours after operation. Note the diminution in the initial downward deflection
and depression of S–T interval. (*D*) Three days after the operation; note the
still further diminution in the initial downward deflection and the high amplitude
of T waves referred to in the text. (*E*) 5 days after operation. Note the small
amplitude of initial downward deflection and change in characteristics of the T
wave. (*F*) 11 days after operation. The initial downward deflection has in-
creased somewhat in amplitude and the S–T interval deviation has also dis-
appeared (*G*) 6 months after operation (chronic stage). The initial down-
ward deflection is small compared to the upward deflection.

of the upward deflection.   Tall upright T waves were observed as a transient phenomenon from the 3d to the 7th day after operation.

This animal was sacrificed after 4 months, and showed the most extensive infarction observed in any of our experiments, involving the lower half of the anterior portion of the left ventricle including the apex and extending into the lateral walls.
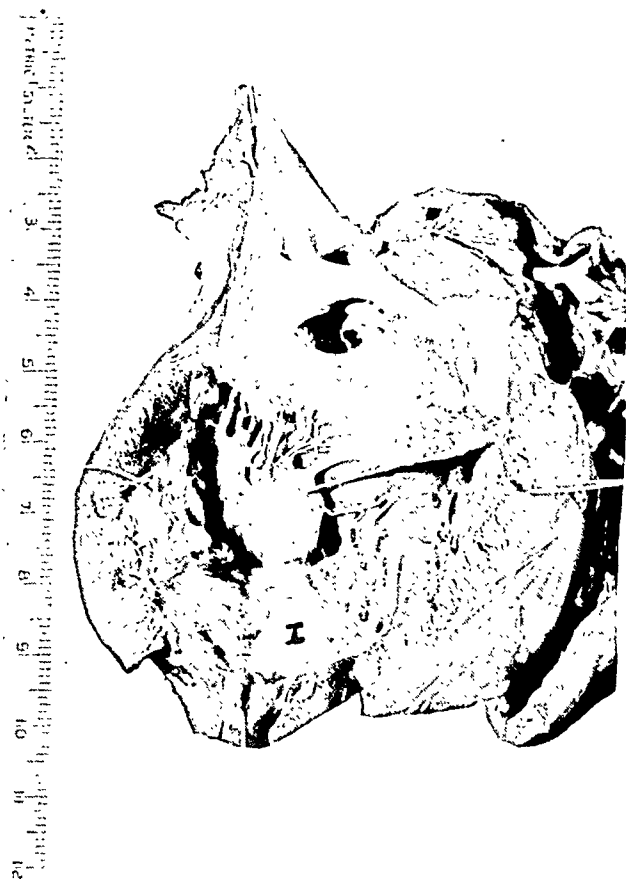
FIG. 6.   SHOWING HEART OF DOG NUMBER 261, THE ELECTROCARDIOGRAMS OF WHICH ARE SHOWN IN FIGURE 5

This animal was sacrificed six months after operation.   Note the area of chronic infarction involving the apex and lower third of anterior wall of left ventricle.

Dog Number 324.   The initial downward deflection was somewhat diminished after operation but was never abolished.   Its amplitude remained about half that of the upward deflection except for one day (21st day after operation) when it exceeded the latter.   On the 42d day after operation its amplitude was about one-third that of the upward deflection. The transient appearance of tall T waves was observed in this case also on the 3d day following operation.

This animal died of bronchopneumonia 3 months after operation. The heart showed moderate pericardial adhesions involving the anterior and lateral portion of the left ventricle. Upon injecting the coronary arteries with an opaque mass the left descending branch remained almost empty.
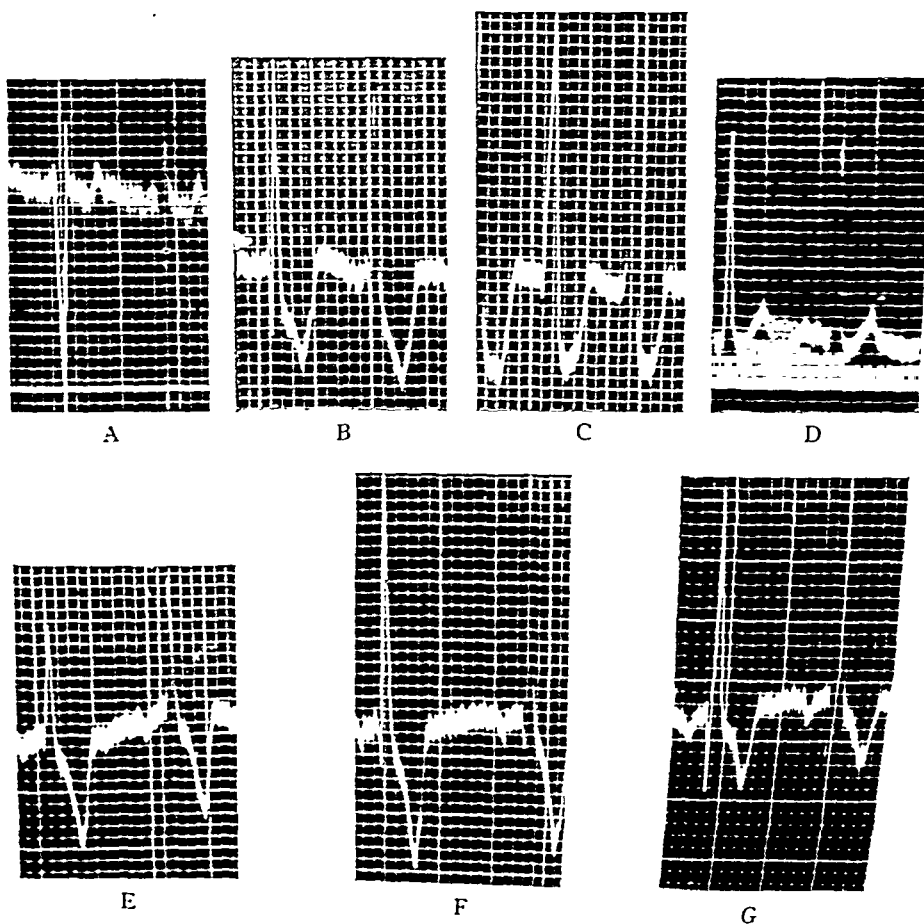
Fig. 7.   Shows the Electrocardiograms from Precordial Lead IV from the Survival Experiment on Dog Number 328

(A) Before operation.   (B) 3 hours after ligation of the anterior descending branch of the left coronary artery. Note the marked diminution in the initial downward deflection and depression of the S-T interval. (C) 24 hours after operation. Note that the initial downward deflection is almost absent and that the S-T interval is situated below the iso-electric line. (D) 4 days after operation. Note the complete absence of initial downward deflection and the upright T wave. (E) 13 days after operation. Note the complete absence of initial downward deflection and the inverted T wave. (F) 27 days after operation. Note the slight initial downward deflection (2 mm.). (G) 6 months after operation. Note the increase in amplitude in the initial downward deflection now measuring 6 mm.

The slight amount of filling that resulted was due apparently to anastomosis from neighboring vessels. The area of infarction was small, involving only an area about two centimeters in diameter on the anterior surface of the left ventricle near the apex. There was no marked thinning of the left ventricular wall.

Dog Number 330. No definite diminution to the initial downward deflection occurred in the first 24 hours following operation. Thereafter, all the deflections were small but the initial downward deflection was especially reduced, being almost absent on the 5th day. By the 37th day the initial downward deflection had practically returned nearly to its preoperative amplitude.

This animal when sacrificed four months after operation showed only a small area of infarction about one and one-half centimeters in diameter involving the anterior portion of the left ventricle near the apex.

Dog Number 328 (Fig. 7). The initial downward deflection gradually disappeared following the operation and was almost absent 24 hours afterwards. It remained almost or entirely absent for about 14 days and then gradually reappeared. Six months after operation it had about one-third the amplitude of the upward deflection. Transient tall T waves were also observed in this animal.

This animal was sacrificed six months after operation. The cardiac findings were similar to those of Dog Number 261.

Cat Number 1. The electrocardiogram taken 135 minutes after ligation of the anterior descending branch of the left coronary artery showed complete loss of the initial downward deflection in Leads IV and V, and an R–T interval deviation situated below the iso-electric line. Subsequent tracings showed a return of the R–T interval to the iso-electric line but the initial downward deflection was absent in the last tracing taken 28 hours after ligation.

The heart showed a marked area of softening involving the lower half of the anterior and lateral portions of the left ventricle.

<div align="center">DISCUSSION</div>

Comparison of electrocardiograms of precordial leads in man and in dogs and cats following coronary occlusion. The initial downward deflection from precordial leads, which in man is either markedly diminished or entirely absent after occlusion of the anterior descending branch of the left coronary artery, is preserved and undergoes almost no change in dogs and cats immediately following ligation of the anterior descending branch of the left coronary artery. Such a marked difference in response of the human subject and the dog or cat invites explanation. To this end we have considered 3 possibilities as a cause for this variation: first, the difference in extent of the region supplied by the occluded vessel in each instance; second, the variation in time ensuing between the occlusion and the tak-

ing of the tracing; and third, the change in the position of the left ventricle relative to the anterior electrode.

In human cases occlusion of the anterior descending branch of the left coronary artery usually occurs close to its point of origin; the resulting area of infarction, therefore, is usually the apex, adjacent lateral wall of the left ventricle and the lower anterior portion of the ventricular septum. The blood supply of the human heart differs considerably from that of the dog and cat. In man the anterior descending branch of the left coronary artery supplies an area corresponding to that which is supplied by the anterior descending, septal and a considerable portion of the circumflex branches of the left coronary artery in the dog and cat. Before comparing the electrocardiographic results in animals with those obtained in man, it is, therefore, important to reproduce as closely as possible in the experimental animal the location and relative size of the infarcted area.

From the standpoint of time, the electrocardiographic findings obtained immediately after experimental occlusion in animals cannot justly be compared with those of the human subjects, in whom, even under advantageous conditions, tracings are rarely taken until many hours after the occlusion has occurred.

Furthermore, since we have evidence that the initial downward deflection seen in the precordial leads is produced chiefly by the left ventricle (see page 740), we regard the position of the left ventricle in relation to the anterior chest wall as not unimportant. The fact that a considerably larger portion of the left ventricle presents anteriorly in the dog and cat than in man may account for the greater difficulty in causing, in the former, the disappearance of the initial downward deflection.

Disappearance of initial downward deflection in acute experiments the result of ligation of multiple coronary arteries and of extensive injury of heart muscle by cauterization. The endeavor to cause infarction of an area of the dog's heart corresponding to that which results in the human heart after occlusion of the left descending branch at its origin was unsatisfactory for our purpose since dogs withstand multiple ligation of the large coronary arteries poorly, the animals usually dying within a few minutes after the last vessel has been tied. As already shown, we were unable during the short period of their survival to cause disappearance of the initial downward deflection in dogs by ligating multiple arteries. In cats, which withstand multiple ligation of coronary arteries relatively well, ligation of the anterior descending branch of the left coronary artery plus ligation of the anterior branch of the circumflex (together supplying the apex, anterior and lateral wall of the left ventricle and a portion of the ventricular septum) resulted in a disappearance of the initial downward deflection. *This indicates that ultimate electrocardiographic changes depend upon the size and location of the infarct produced rather than upon the ligation of any particular vessel.*

Cauterization of the heart muscle in dogs and cats also resulted in a marked diminution or disappearance of the initial downward deflection. The surface which had to be cauterized, however, to bring about this result was relatively much more extensive in dogs than in cats. In the dog cauterization of the apex and lower part of the left ventricular wall, while interfering to some degree with the circulation of the underlying muscle, does not eliminate the possibility of the muscle of the damaged area receiving considerable nourishment from vessels in the neighborhood. In acute experiments it is apparently only when the circulation from these surrounding areas is also impaired that the initial downward deflection is either markedly diminished or entirely eliminated. Because of the smaller size of the cat's heart, cauterization, although not as extensive as in the dog with respect to area of surface, really involves a greater number of vessels and relatively greater depth of muscle.

In acute experiments the disappearance of the initial downward deflection was brought about in cats (1) after ligation of the anterior descending branch plus the anterior branch of the circumflex and (2) after cauterization of an *extensive* area of the anterior portion of the left ventricle. In dogs, the same effect was brought about only with the greatest difficulty. Ligation of vessels proved ineffectual; the only successful procedure was the cauterization of a massive portion of the heart muscle, relatively much more than is involved in the infarction observed in the human heart at necropsy.

In survival experiments, however, the initial downward deflection almost or entirely disappeared in three dogs and in one cat 24, 24, 48 and 2 hours respectively after ligation of the anterior descending branch of the left coronary artery. In two additional dogs moderate diminution of this deflection was produced. In the former group frequent electrocardiograms showed gradual decrease in size of the initial downward deflection beginning soon after operation, and progressing until it either finally disappeared or remained with an amplitude of only one to two mm. Only the left descending branch of the coronary artery was ligated in these experiments. The question arises as to why the disappearance of the initial downward deflection after ligation was gradual. We suggest that it was so because the maximum degree of cardiac damage or cardiac infarction accompanied by actual death of muscle probably developed only 24 to 48 hours after ligation of the anterior descending branch of the left coronary artery.

The observations in the survival experiments are quite similar to those in man if the time interval ensuing between occlusion and the taking of the electrocardiogram is taken into consideration. The records observed in the dogs 24 hours after ligation of the anterior descending branch of the left coronary artery are quite similar to those in man after occlusion of this vessel; in fact, it is almost impossible to distinguish typical records. In

most instances, the disappearance of the initial downward deflection is not as complete in the dog as in man. The tracing taken 24 to 48 hours after ligation in the dog often shows a slight downstroke of 1 to 2 mm. preceding the upstroke, whereas in man the downstroke is in most instances entirely absent at a similar interval following occlusion.

The return of the initial downward deflection more completely and more rapidly in dog than in man may be explained in several ways: (a) that a relatively smaller vessel was occluded in the dog than occurs in patients and that the resulting infarct was, therefore, relatively smaller; (b) that in the dog, the remaining vessels were normal and were, therefore, capable of furnishing the maximum of collateral circulation, whereas in patients marked atheroma is often present in the other coronary arteries; (c) that in the dog, the vessel distal to the ligation being normal may still function to some degree with the development of anastomoses, whereas in patients frequently the presence of atheroma of the vessels distal to the point of thrombosis with consequent slowing of the blood current may lead to occlusion below the point of initial thrombosis.

The presence of the high amplitude of the T wave in precordial leads which was observed as a transient phenomenon in all of our dogs in the subacute stage of infarction is almost identical with that observed in man (13) (15). In the latter it has been observed as long as 2 to 4 weeks after the onset of the acute infarction. Its presence is of considerable importance. Bohning and Katz (2) have recently reported the presence of such T waves in human indirect leads; and Wood and Wolferth have reported their presence in precordial leads (15).

Cause of production and disappearance of initial downward deflection of precordial leads. The configuration of the electrogram from the ventricular surface and of the electrocardiogram from the precordial leads is quite similar (7); the higher voltage in the former is due the electrode being placed directly upon the heart, whereas in the latter, it is placed at some distance from the heart. The electrogram, when the electrode on the heart is placed over the normal muscle, shows an initial downward deflection diminishing as the great vessels are approached (5) (10). This relation also holds for the electrocardiograms from the precordial leads. From these findings it would appear that when in the precordial leads a large electrode is used (i.e., one covering the entire precordium), the electrocardiogram may be considered as due to the summation of the action currents from the muscles of the entire heart; where a small electrode is used the electrocardiogram is produced chiefly by action currents from the muscle lying immediately beneath the electrode and only to a lesser degree by those from muscle more distant. Since the voltage recorded is probably much greater from the left ventricle than from the right it is probably the former which largely determines the ventricular complex in the precordial leads. For example in the experiment (Fig. 3) in which the apex and

lower third to half of the anterior wall of the left ventricle was cauterized and the electrogram from the cauterized area showed a disappearance of the initial downward deflection formerly present, the initial downward deflection was preserved in the electrogram of the uninjured muscle of the right ventricle. The precordial lead (Lead IV), using a large electrode covering the entire precordium, showed a complete absence of the initial downward deflection. The same was true in survival experiments in the dog and in human cases in which the infarction involved only the lower anterior half of the left ventricle while the right ventricle was intact.

From these data it would appear that we have information regarding the portions of the heart responsible for determining the presence or absence of the initial downward deflection in precordial leads. The important area appears to be the apex, lower anterior and lateral walls of the left ventricle and probably in addition the lower anterior and left side of the interventricular septum.[3] The chief evidence that we have obtained supporting this statement is: (1) the absence of the initial downward deflection from precordial leads in 12 human subjects in whom infarction had involved this area; and (2) the disappearance of the initial downward deflection after experimental ligation of vessels that supply this area or after cauterization of the heart muscle of this region.[4]

SUMMARY AND CONCLUSIONS

In acute experiments upon dogs and cats ligation of the anterior descending branch of the left coronary artery, while causing R–T deviation in precordial leads produced practically no change in the initial downward deflection of the ventricular complex. In general, ischemia or damage to a much greater portion of the myocardium than that supplied by the anterior descending branch of the left coronary artery was required to produce a marked diminution or disappearance of the initial downward deflection. Diminution or disappearance of this deflection was produced in cats after ligation of the anterior branch of the left coronary plus the anterior branch of the circumflex arteries; or in dogs and cats after cauterization of a considerable portion of the surface of the cardiac muscle.

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[3] In the human cases, infarction of the lower anterior portion of the septum is nearly always present after occlusion of the left descending branch; in dogs, involvement of the septum does not occur unless the septal branch is ligated in addition to the anterior descending branch of the left coronary artery.

[4] Wilson (11) has pointed out the similarity of the electrocardiogram from precordial leads in cases of infarction of the anterior surface of the left ventricle to the electrogram obtained when the anterior electrode is placed at the pulmonic area just above the pulmonary valves or at other points that lie opposite one of the valvular orifices. He suggests that the early occurrence of a prominent upstroke not preceded by a preliminary downstroke in these curves may be due to the presence of a large infarct which, being composed of dead and inactive muscle, has an effect upon the electrocardiogram similar to that which might be produced by a window or orifice in the anterior wall of the heart.

In survival experiments marked diminution or disappearance of the initial downward deflection could be produced by ligation of the anterior descending branch of the left coronary artery alone. In 3 out of 5 dogs this deflection almost completely disappeared 24 to 48 hours after ligation. In one cat it disappeared 2 hours after operation. In one dog it was markedly diminished on the 5th day after operation and in another moderately diminished on the 3d day. In all 5 dogs, later during the recovery stage, it increased again; in one instance the return was complete, in the remaining 4, the deflection regained one-fourth to one-half its original amplitude. In man the initial downward deflection which disappears during the acute stage of infarction usually does not return in the chronic stage; occasionally, however, a partial return occurs. The return of this deflection in the dog is probably to be explained by the smaller relative size of the area involved by the chronic infarct and by the larger portion of the left ventricle in relation with the anterior chest wall in the dog.

Extremely tall T waves observed in these experiments as a transient phenomenon are believed to characterize a subacute stage of infarction.

Ligation of the coronary arteries supplying the posterior wall of the left ventricles or injury of this area by cauterization produced R–T interval deviations above the iso-electric line but no change in the amplitude of the initial downward deflection.

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THE INFLUENCE OF PROTEIN INTAKE ON THE UREA CLEARANCE IN NORMAL MAN

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Cope (1) has reported that a change in protein content of the diet from 75 grams to 40 grams per day is accompanied by a lowering of the urea clearance in nephritics, who had approximately normal urea clearances during the control period. No doubt Cope's investigations, like our own, were stimulated in part by the observations of Jolliffe and Smith (2) that in dogs the urea clearance may be raised by over 100 per cent by change from a low protein to a high protein diet. Since the effect of diet in Cope's experiments appears to be much less marked than is the case in the dog, it was felt that the problem merited further investigation in normal man to avoid the possibility of overlooking a functional response that might be depressed in a structurally diseased kidney. Consequently we have repeated these experiments upon subjects without renal, cardiac or vascular diseases and with no recent febrile reaction (3). As a further modification of Cope's experiments the protein intake has been varied from 9 grams to 280 grams per day, and in most instances the rate of urine excretion has been above the augmentation limit (2 cc. per minute).

The subjects were volunteers selected from the wards of the Third (New York University) Medical Division of Bellevue Hospital. Urea clearances were performed in the morning without breakfast, with the patient recumbent in bed. During the previous night and up to two hours before the discard preceding the first urine collection period, the subject was given 3,000 to 4,000 cc. of water to insure high rates of urine flow. An effort was made to avoid collection of urine in the ascending phase or at the peak of diuresis. All discards and urine specimens were collected by catheterization to insure complete emptying of the bladder. Blood was drawn from the median basilic vein at the beginning, middle and end of each experiment. When the three blood urea values, as separately determined, checked within three per cent, they were averaged, and when they did not, values interpolated to the middle of each period were used. Urea in plasma and in urine were determined in duplicate by the gasometric

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[1] Graduate Students in the Department of Medicine.

TABLE I

Data of urea clearances

	Daily protein intake	Date	V Urine volume	P Plasma urea	$\frac{UV}{P(SA)}$ clearance
	grams		*cc. per minute*	*mgm. per cent*	*cc. per sq. m. per minute*
M. O., age 40, surface area 1.7 sq. m.	100	November 7, 1933	3.59	18.9	38.9
			5.00	19.0	57.0
			8.31	19.0	58.0
			3.56	18.99	44.1
		November 9, 1933	17.29	21.15	47.8
			16.50	21.39	40.4
			15.20	21.05	49.1
			11.35	20.07	44.5
	Average				47.5
	9	November 15, 1933	12.07	5.93	34.6
			13.68	6.22	38.9
			13.07	6.15	39.1
			12.50	6.10	36.6
		November 17, 1933	12.00	6.95	36.4
			11.46	6.52	37.4
			11.16	6.51	32.8
			11.80	6.50	35.3
	Average				36.3
	280	November 23, 1933	19.00	37.89	53.0
			15.70	37.69	34.8
			15.75	37.05	47.2
			14.80	37.55	50.0
		November 25, 1933	15.70	34.10	44.8
			16.00	33.61	51.9
			13.40	33.30	43.2
			13.27	32.96	46.1
		November 28, 1933	18.25	41.30	48.2
			17.15	39.80	49.5
			14.90	38.70	43.6
			14.72	37.90	45.3
	Average				46.4
P. M., age 48 years, surface area 1.84 sq. m.	100	December 6, 1933	4.28	23.90	54.6
			4.28	23.50	56.8
			5.48	23.38	55.1
			7.71	23.28	61.5
		December 8, 1933	4.52	25.86	57.4
			5.12	25.80	59.4
			3.82	25.65	50.1
			2.60	25.50	42.9
	Average				54.8

method of Van Slyke (4). Heparin was used as an anticoagulant. All patients were attended by a special nurse and the diets were prepared by a trained dietician attached to the teaching unit of New York University Medical Service.

The control urea clearances were determined while patients were receiving the usual ward diet containing an average of 100 grams of protein

TABLE I *(continued)*

	Daily protein intake	Date	V Urine volume	P Plasma urea	UV / P(SA) clearance
P. M. *(continued)*	*grams*		*cc. per minute*	*mgm. per cent*	*cc. per sq. m. per minute*
	9	December 13, 1933	3.73	9.05	43.6
			4.64	8.62	54.2
			4.23	8.60	47.7
			3.07	8.50	38.2
		December 15, 1933	4.86	9.15	48.3
			3.20	9.15	46.9
			3.10	9.15	44.3
			3.30	9.15	42.5
	Average				45.6
	280	December 20, 1933	2.75	41.37	48.0
			3.70	40.84	55.3
			4.60	40.32	56.3
			4.63	39.83	56.1
		December 22, 1933	6.04	38.60	59.6
			4.80	38.94	62.3
			5.08	38.45	57.4
			3.43	38.05	55.2
	Average				56.0
J. G.,† age 37 years, surface area 1.66 sq. m.	9	December 16, 1933	6.41	8.42	31.7
			4.23	8.42	32.5
			3.37	8.42	28.6
			3.50	8.42	30.4
	Average				30.8
	280	December 22, 1933	11.35	47.35	33.2
			10.45	46.70	40.3
			7.36	46.30	38.6
			3.06	45.90	30.1
	Average				35.5
	100	December 29, 1933	12.65	24.05	34.2
			12.35	24.30	38.5
			10.90	23.75	36.7
			9.18	23.35	34.3
	Average				35.9

per day.   Eight clearances were determined on two different days.   Following the last control observation the patient was given a diet containing 9 grams of protein per day.   On the sixth day four clearances were determined, and on the eighth day, four more.   The same procedure was fol-

TABLE I *(continued)*

	Daily protein intake	Date	V Urine volume	P Plasma urea	$\frac{UV}{P(SA)}$ clearance
	grams		*cc. per minute*	*mgm. per cent*	*cc. per sq. m. per minute*
M. C., age 40 years, surface area 2.08 sq. m.	100	January 1, 1934	4.67	18.70	28.5
			5.50	18.05	44.1
			5.13	17.95	36.4
			5.20	17.90	35.0
		February 2, 1934	23.05	26.90	42.2
			20.95	26.35	39.6
			21.20	26.20	41.4
			20.60	26.00	42.1
	Average				38.6
	9	February 7, 1934	6.77	8.54	24.5
			6.10	8.83	24.8
			6.54	9.01	31.5
			4.80	9.26	23.6
		February 9, 1934	5.63	6.57	25.4
			4.95	6.57	23.6
			4.08	6.57	21.1
			4.00	6.57	22.0
	Average				24.6
	280	February 14, 1934	4.52	38.40	44.8
			6.73	38.40	45.4
			2.85	38.40	30.5
			2.64	38.40	34.9
		February 16, 1934	5.80	41.18	29.7
			2.45	39.95	25.6
			2.69	39.78	33.1
			5.35	39.43	46.7
		February 21, 1934	6.57	39.50	33.1
			3.17	39.50	34.3
			3.15	39.50	33.1
			2.62	39.50	32.4
		February 28, 1934	2.73	36.61	31.9
			4.77	35.90	43.9
			7.73	35.63	45.4
			6.87	35.23	36.2
		March 5, 1934	1.73	40.40	21.9*
			1.55	40.40	25.7*
			1.80	40.40	27.4*
			1.74	40.40	27.1*
	Average				36.3

lowed on the high protein diet which contained 280 grams of prote⟨...⟩ protein in this diet was composed principally of meat, cheese ⟨...⟩ products, meat constituting about one-third. On one subject (⟨...⟩ clearances were determined on a high protein diet over a period o⟨...⟩ five days. A total of 124 urea clearances were determined on ⟨...⟩ jects, and with five exceptions the urine flow was above 2 cc. pe⟨...⟩ The results of these observations are given in Table I.

The greatest reduction in clearance observed on the low pro⟨...⟩ was 36 per cent below the control, and the average reduction wa⟨...⟩ cent. We have no doubt that this change is significant and re⟨...⟩

TABLE I (*continued*)

	Daily protein intake	Date	V Urine volume	P Plasma urea	⟨...⟩
J. J., age 45 years, surface area 1.68 sq. m.	*grams* 100	February 26, 1934	*cc. per minute* 5.42	*mgm. per cent* 19.70	35.5
			2.93	19.50	32.5
			2.30	19.30	36.1
			2.64	19.20	43.5
		March 2, 1934	3.27	17.70	36.3
			2.85	17.70	32.5
			2.086	17.70	30.3
			1.76	17.70	22.4
	Average				
	9	March 7, 1934	11.00	5.76	
			8.14	5.10	
			5.91	5.18	
			3.68	5.28	
		March 9, 1934	12.10	7.47	
			10.42	7.43	
			10.10	6.91	
			8.00	6.47	
	Average				
	280	March 14, 1934	2.75	44.40	
			3.23	43.10	
			2.36	42.10	
			1.92	40.80	
		March 16, 1934	2.53	46.42	
			2.45	45.98	
			2.42	45.08	
			2.53	44.22	
	Average				

* Calculated as " Standard " urea clearance but not included in ave⟨...⟩
† Note different order in which diets were given.

altered activity of the kidney in the excretion of urea. Increasing the protein intake above normal, however, did not result in a significant increase in clearance. In the one subject on whom protracted observations were made, the average urea clearance after 25 days on the high protein diet was slightly less than on the control diet.

Our observations on normal man confirm Cope's observations on nephritics with approximately normal urea clearances: the urea clearance is not significantly raised by a high protein diet, although it is significantly lowered by a low protein diet. Our observations show further that the lowering of the urea clearance is of the same order of magnitude, whether the clearances are determined above or below the augmentation limit. No explanation can be advanced for the fact that renal activity in man is so much less susceptible to dietary influence than is the case in the dog; it may be pointed out, however, that Jolliffe and Smith fed much larger quantities of protein (15 grams per kilogram of body weight) than our subjects consumed (4 grams per kilogram of body weight) and that in the experiments on the dog all the protein was supplied as meat whereas in man about one-third of the total protein intake is the most that can be conveniently ingested in this form.

SUMMARY

Observations on the urea clearance at urine flows above the augmentation limit in normal men subsisting on diets containing 9 or 280 grams of protein per day show a reduction in clearance at the lower protein level, but no change in clearance at the higher protein level as compared with a control period when the protein intake was 100 grams per day. These results are in agreement with Cope's observations on nephritics with approximately normal urea clearances, which observations were made, for the most part, when the urine flow was below the augmentation limit.

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THE EFFECTS ON RENAL ACTIVITY OF THE ORAL
ADMINISTRATION OF PHLORIZIN IN MAN

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Chasis, Jolliffe and Smith (1) have shown that phlorizin administered intravenously to man, raises the level of the glucose clearance to that of the simultaneous xylose and sucrose clearances, the duration of this effect depending upon the size of the dose. In the largest doses given, the creatinine clearance was not significantly affected with respect to the clearances of the non-metabolized sugars.

It appeared desirable to investigate the effects of phlorizin when administered by mouth. It seemed possible that, if the phlorizin were well absorbed, complete glycuresis might be maintained for considerable periods of time by the repeated administration of small doses and that under these conditions the clearances of creatinine, glucose and xylose might be brought together, as happens in the dog after larger doses of phlorizin (2). Chasis, Jolliffe and Smith (1) have reviewed the literature on the oral administration of phlorizin in man, but in none of this previous work have the recorded observations made it possible to evaluate the effects upon the glucose clearance.

Volunteers for this study were obtained from the Third (New York University) Medical Division of Bellevue Hospital. During the period of observation they were segregated and under the care of a special nurse. The subjects were maintained on the regular ward diet and the phlorizin tests were performed before breakfast, with the subject recumbent in bed. Blood was drawn from the antecubital vein, heparin being used as an anticoagulant. The blood was centrifuged and the plasma precipitated immediately. All urine discards and observation specimens were removed by catheter and collected in flasks containing a few benzoic acid crystals. Appropriate dilutions were made subsequently, and plasma and urine were analyzed by the methods of Jolliffe, Shannon and Smith (3).

The usual procedure was as follows: 100 grams of xylose and 10 grams of creatinine were mixed with about 10 tablespoons of oatmeal or dissolved in 200 cc. of water. This was taken by the subject at 7:30 a.m.; at 9 a.m. the bladder was catheterized for the first discard and immediately there-

TABLE I

Simultaneous xylose, glucose, urea and creatinine clearances in man before and after oral administration of phlorizin

Subjects	Surface area	Phlorizin	Time from phlorizin	Number of periods averaged	Average urine volume	Average clearance				Clearance ratios		
	square meters	*mgm. per kilo*	*minutes*		*cc. per minute*	Xylose	Urea	Creatinine	Glucose	Urea / Xylose	Creatinine / Xylose	Glucose / Xylose
						cc. per sq. m. per minute						
M. O'L.	1.7		Control	2	7.60	48.7	34.3	106.0		0.70	2.18	
		150	18–95	3	2.02	40.7	25.3	77.6	30.8	0.62	1.90	0.76
			Control	2	2.30	52.4	38.4	101.8		0.73	1.94	
		200	160–250	4	1.57	36.5	23.3	66.8	18.4	0.64	1.83	0.50
M. C.	2.08		Control	2	1.90	55.3	31.1	90.4		0.56	1.63	
		400	130–250	4	1.88	33.3	14.4	47.3	32.6	0.43	1.42	0.98
			Control	2	2.54	52.2	31.8	96.5		0.61	1.85	
		400	240–350	4	1.73	40.2	20.1	61.6	21.7	0.50	1.53	0.54

after the first blood sample was drawn. At 9:15 the first urine sample was collected. At 9:30 a.m. the second urine sample and the second blood were obtained. Two control periods being completed, repurified phlorizin was administered as a suspension in water, or where feasible, as a solution in whiskey. At 10 a.m. the bladder was catheterized for the second discard (washout) and the third blood sample was drawn. At 10:15 a.m. and 10:30 a.m. respectively the third and fourth urine samples were removed by catheter. At 10:35 a.m. the fourth blood sample was drawn. At 10:45 a.m. the fifth urine sample was obtained. At 11 a.m. the sixth urine sample and the fifth blood were obtained, completing the experiment. From the first two values for urea, creatinine, glucose and xylose in blood an interpolation was made to the midpoints of the two control periods and from the last three values interpolations to the midpoints of the four test periods.

It was found in two preliminary series of observations in which 50 mgm. and 100 mgm. of phlorizin per kgm. (dissolved in whiskey) were administered, that the resulting glycuresis reached a maximum within 30 minutes and disappeared within 6 to 7 hours. It was apparent that anything approaching complete glycuresis could only be obtained by the repeated administration of fairly large doses. A summary of glucose, xylose and creatinine clearances observed simultaneously is given in Table I. In the first instance the phlorizin was given in a single dose; in the others in four divided doses at approximately 45 minute intervals. The quantity of phlorizin administered in the last two instances (four doses of approximately 8 grams each) is all that can be taken conveniently by stomach because of the large volume of fluid required. It is clear that this quantity of phlorizin is inadequate to raise the glucose clearance to the level of the xylose clearance with any certainty, and it is concluded that although partial glycuresis can be obtained readily for short periods of time by small doses of phlorizin administered orally, it is practically impossible to obtain complete glycuresis by this procedure.

Our data show that the clearances of urea, creatinine and xylose are all depressed after large doses of phlorizin, indicating a reduction in glomerular activity such as was observed after the intravenous administration of this drug in man by Chasis, Jolliffe and Smith (1), in the dog by Shannon, Jolliffe and Smith (2) and in the sculpin (in which the glomeruli may be completely closed by large doses of phlorizin) by Marshall and Grafflin (4). It would appear that this effect upon glomerular activity is a characteristic though rather variable feature in the physiological action of this drug. This depressant effect upon glomerular activity, and transient nausea with the larger doses, were the only unfavorable symptoms noted in these observations. In all instances the urine was free from sugar and otherwise normal for several days afterward. Our results do not warrant the belief that the administration of phlorizin by mouth over mod-

The material will be presented briefly as follows: 1, chemical methods; 2, endogenous blanks; 3, experiments; 4, constants of the functions; 5, analysis of errors; and 6, the excretion constant of xylose.

CHEMICAL METHODS

The details of the methods of collection and preservation of plasma and urine are given in a previous publication (1). Duplicate and sometimes triplicate analyses were made on the plasma and urine samples, all of which had been renumbered by an independent worker so as to obviate bias. The Folin and Wu (4) method with d-xylose (Pfanstiehl) standards of appropriate strength was used for the determination of the reducing substances of urine and the nonfermentable reducing substances of plasma. For color matching a Klett Biocolorimeter with north light was used. The intensity of illumination was greatly cut down when the strength of dilute solutions was determined (5). It was found under the conditions observed that the reducing power of plasma and urine varied insignificantly during a four day period, the maximum time for a complete series of analyses for an experiment.

For the removal of the yeast-fermentable reducing substances of plasma the method of Blanco (6) was found to yield the most consistent results and, within the limits of experimental error, complete recovery of added xylose. In every series of plasma determinations, yeast blanks were run as a check on the washing of the cells. Consistent results were obtained throughout with tungstic acid extracts of plasma and yeast standing at room temperature for from 15 minutes up to 2 hours before centrifuging and decanting the supernatant fluid. The total amount of reducing substances in plasma and yeast, before the ingestion of xylose, will be regarded as the blank, and its mean value from a number of determinations will be called the endogenous blank of plasma concentration.

The reducing substances of urine gave less consistent values when yeast was used, although added xylose was completely recovered. Consequently the determination of reducing substances was made without yeast on urines diluted with distilled water so that 1 cc. of the final dilution, the minimum amount pipetted, contained no more than 0.4 mgm. of reducing substances.

Endogenous blanks. The total nonfermentable reducing substances of oxalated plasma plus yeast were, for Subject E, mean of 7 determinations, 6.46 mgm. per 100 cc., standard error of mean 0.9989; for Subject D, mean of 7 determinations, 6.87 mgm. per 100 cc., standard error of mean 0.7144.

The output of reducing substances in urine were, for Subject E, mean of 19 determinations, 0.9037 mgm. per minute, standard error of mean 0.0373; for Subject D, mean of 9 determinations, 1.0595 mgm. per minute, standard error of mean 0.0474. There is no significant difference between

the two subjects as far as their endogenous blanks for plasma concentration are concerned, but the difference between their endogenous outputs of reducing substances, small as it is, can be shown to be statistically significant.

EXPERIMENTS

The protocols are given in Table I. In all the experiments diarrhea of varying degree developed, more marked with the larger amounts ingested.

TABLE I

Experimental data on xylose excretion

Experimental details	Time of urine collection	Urine volume	Urine xylose	Time of blood collection	Plasma xylose
		cc.	*mgm. per 100 cc.*		*mgm. per 100 cc.*
Subject E, female, white; body weight	8.24– 9.27	163	37.6	9.23	4.5
45 kgm.; standing height 159 cm.	9.27–10.09	131	205	10.01	16.1
Experiment E-1, November 15, 1933.	10.09–10.39	28	2823	10.31	44.8
50 grams xylose, 9.28 a.m.; break-	10.39–11.38	53	3545	11.33	62.2
fast, 7.20 a.m. Routine labora-	11.38–12.38	69	3940	12.25	69.5
tory work during this and subse-	12.38– 1.44	57	4380	1.30	49.6
quent experiments. d-xylose,	1.44– 2.43	38	3895	2.32	31.6
Pfanstiehl, by mouth in all experi-	2.43– 3.51	36	3293	3.34	25.4
ments.	3.51– 4.48	25	2898	4.35	21.3
	4.48– 5.44	19	2380	5.37	13.9
Experiment E-2, December 4, 1933.	7.30– 8.10	219	22	8.36	4.9
25 grams xylose, 8.45 a.m.; break-	8.10– 8.43	179	20	9.45	34.5
fast, 7.15 a.m.	8.43– 9.50	122	747	10.43	35.1
	9.50–10.49	437	360	11.32	29.7
	10.49–11.38	380	275	12.29	18.3
	11.38–12.29	264	325	1.25	13.3
	12.29– 1.35	320	149	2.33	12.1
	1.35– 2.21	476	54.8	3.46	7.6
	2.21– 2.53	331	40.8	4.35	8.1
	2.53– 3.35	363	37.8		
	3.35– 4.09	257	31.1		
	4.09– 4.44	235	30.2		
Experiment E-3, January 10, 1934.	8.08– 9.07	99	61		
25 grams xylose, 9.08 a.m.; break-	9.07–10.14	47.5	1768		
fast, 7 a.m	10.14–11.10	55.5	3258		
	11.10–12.15	106	1824		
	12.15– 1.17	124	1010		
	1.17– 2.17	52	1295		
	2.17– 3.17	149	287		
	3.17– 4.14	213	133		
	4.14– 5.14	32	566.5		

TABLE I (continued)

Experimental details	Time of urine collection	Urine volume	Urine xylose	Time of blood collection	Plasma xylose
		cc.	mgm. per 100 cc.		mgm. per 100 cc.
Subject D, male, white; body weight 70 kgm.; standing height 179 cm. *Experiment D-1, November 23, 1933.* 50 grams xylose, 8.59 a.m.; breakfast, 8.15 a.m.	7.50– 8.55	46.5	158	8.53	5.3
	8.55– 9.59	98.5	312	9.58	84.4
	9.59–10.56	97.5	3605	10.55	91.8
	10.56–11.55	100	3235	11.52	69.4
	11.55– 1.14	82.5	2963	1.10	38.1
	1.14– 2.06	36.5	2410	2.03	20.8
	2.06– 3.13	42.5	1736	3.05	14.9
	3.13– 4.09	28.5	1353	4.07	13.0
	4.09– 5.15	27	1163	5.07	10.8
Experiment D-2, December 13, 1933. 25 grams xylose, 9.32 a.m.; breakfast at 8.30 a.m. Part of the urine of the fourth period was lost before the volume was measured. The volume estimated from the creatinine concentration is 158.4 cc.	8.28– 9.30	71.5	110	9.34	5.6
	9.30–10.41	144.5	1475	10.40	65.2
	10.41–11.43	219	904	11.42	43.9
	11.43–12.36		914	12.35	37.0
	12.36– 1.46	106	1064	1.45	19.0
	1.46– 2.39	85.5	511	2.37	16.3
	2.39– 3.39	49	662	3.37	7.7
	3.39– 4.39	50	420	4.38	9.4
	4.39– 5.39	107.5	144	5.37	7.0
Experiment D-3, January 10, 1934. 25 grams xylose, 10 a.m.; breakfast, 8.30 a.m. Five minutes were lost between the third and fourth periods due to defecation.	7.55– 9.58	174	84.3		
	9.58–11.14	118.5	1639		
	11.14–12.20	143.5	1763		
	12.25– 1.48	152	1592		
	1.48– 2.45	44	1783		
	2.45– 3.47	55	846		
	3.47– 4.39	44	572		
	4.39– 6.15	66	458.5		

Constants of the fitted functions. The fitted functions were:

$$y = y_0 + ae^{-\alpha t}, \tag{1}$$

$$x = x_0 + be^{-\beta t}, \tag{2}$$

where y is the rate of excretion of xylose, in mgm. per minute, x the concentration of xylose in mgm. per 100 cc. of plasma, y_0 and x_0 the endogenous blanks already given, e the base of natural logarithms, t the time in hours, and a, b, α, and β constants to be determined from the data. Inasmuch as the method of calculation is the same as that used for creatinine (1), all details will be omitted. However, there is one point that calls for a brief comment. In one of the experiments it was found impossible to fit the points satisfactorily by least squares applied to the weighted logarithms. It was necessary to go back to the classical method of least squares after reducing the fitting function to a linear form by Taylor's theorem.

The constants α and β are given in Table II. The ratio (α/β) from pairs of simultaneous equations is 0.9975, 0.9621, 0.9022, and 1.1948, mean 1.014, so that α and β are practically equal, which establishes the linearity of the relation between the plasma concentration and the rate of output.

TABLE II

Exponentials fitted to excretion rate (y) and plasma concentration (x) of xylose

$$y = y_e + ae^{-\alpha t}, \qquad x = x_e + be^{-\beta t}$$

Experiment	Amount ingested	$\alpha.\log e$	Number of observations	$\beta.\log e$	Number of observations	$\bar{V}$†
	grams					
D-1	50	0.2297	6	0.2546	6	0.835
D-2	25	0.2754	5	0.2305	7	1.314
D-3	25	0.2868	5			1.005
E-1	50	0.1656*	5	0.1660*	6	0.661
E-2	25	0.2340	8	0.2432	6	7.673
E-3	25	0.2412	5			1.924

Mean $(\alpha.\log e) = 0.254$, mean $(\beta.\log e) = 0.243$.

* Excluded from mean.

† $\bar{V} =$ mean of urine rates for whole experiment, in cc. per minute.

It should be remarked that if the exponentials are made asymptotic to zero (as done by Fishberg (7) as far as the plasma concentration is concerned), the values of α and β would be definitely different from each other, and the resulting relation between y and x would not be linear.

Analysis of errors. From the result of Table II, that a mean value can be obtained for α ($=$ mean β), new values can be computed for a and b, and from these, theoretical values for y and x, let us say, Y and X respectively. The differences $(y - Y)$ and $(x - X)$ are the deviations of y and x from their respective mean curve, and are independent of each other. Experiment E-1 cannot be included in this analysis because of the low value of α (and β). This low value, probably due to delayed absorption, will be considered in a future publication.

Errors of x. Nineteen residuals were obtained with constants and distribution as follows:

Average error	1.6526
Standard deviation (s)	2.2296
Probable error (p.e.)	1.5333
Positive errors	10
Negative errors	9
Number within ± p.e. 11	expected 9.5
Number outside ± p.e. 8	expected 9.5
Number within ± s 15	expected 12.97
Number outside ± s 4	expected 6.03
Number within ± 2s 17	expected 18.14
Number outside ± 2s 2	expected 0.86

The mean is — 0.3684 with standard error = 0.5234, so that the mean is not significantly different from zero.

Errors of y. Twenty-nine residuals were available with constants and distribution as follows:

Average error	0.6796
Standard deviation (s)	0.9134
Probable error (p.e.)	0.6161
Positive errors	13
Negative errors	16
Number within ± p.e. 16 ....expected 14.5	
Number outside ± p.e. 13 ....expected 14.5	
Number within ± s 19 ....expected 19.8	
Number outside ± s 10 ....expected 9.2	
Number within ± 2s 28 ....expected 27.7	
Number outside ± 2s 1 ....expected 1.3	

The mean is —0.0546 with standard error 0.1696. The mean is, therefore, not significantly different from zero.

A comparison with a similar analysis of the creatinine residuals (1) shows that the errors of the data on xylose are larger, especially the errors of the plasma concentration, as was to be expected from the nature of the chemical methods employed. On the other hand, the distribution of the errors of xylose output is considerably closer to the normal distribution of errors, indicating that perhaps idiosyncrasies in one subject have been neutralized by mixing the data of two different subjects.

The excretion constant of xylose. If in the process of fitting, the time of ingestion has been reckoned as the zero-time, Equations 1 and 2 are automatically synchronized. The elimination of the time between these two equations yields the relation,

$$(y - y_c) = A(x - x_c), \tag{3}$$

where $A = (a/b)$ is the excretion constant of xylose.

The value of A from four simultaneous experiments is as follows: D–1, 0.690; D–2, 0.815; E–1, 0.777; E–2, 0.817; mean A, 0.77. In Experiment E–1 the calculation was made directly from the fitted equations, but in Experiments E–2, D–1, and D–2 the constant A was computed after recalculation of a and b (see Equations 1 and 2) using the mean value of $(a . \log e) = 0.25$, or $\alpha = 0.5756$, instead of the values of a or β given in Table II.

It should be realized that, although the errors of the time can be looked upon as negligible as compared with the errors of y or x, it is by no means certain that the synchronization of two pairs of curves insures the simultaneity of the events. Theoretically at least, the y curve lags behind the x curve, because of the time elapsing between the formation of urine and its excretion. Besides, the y points have been computed as loaded ordinates in the middle of the interval of urine collection, but since the fall in
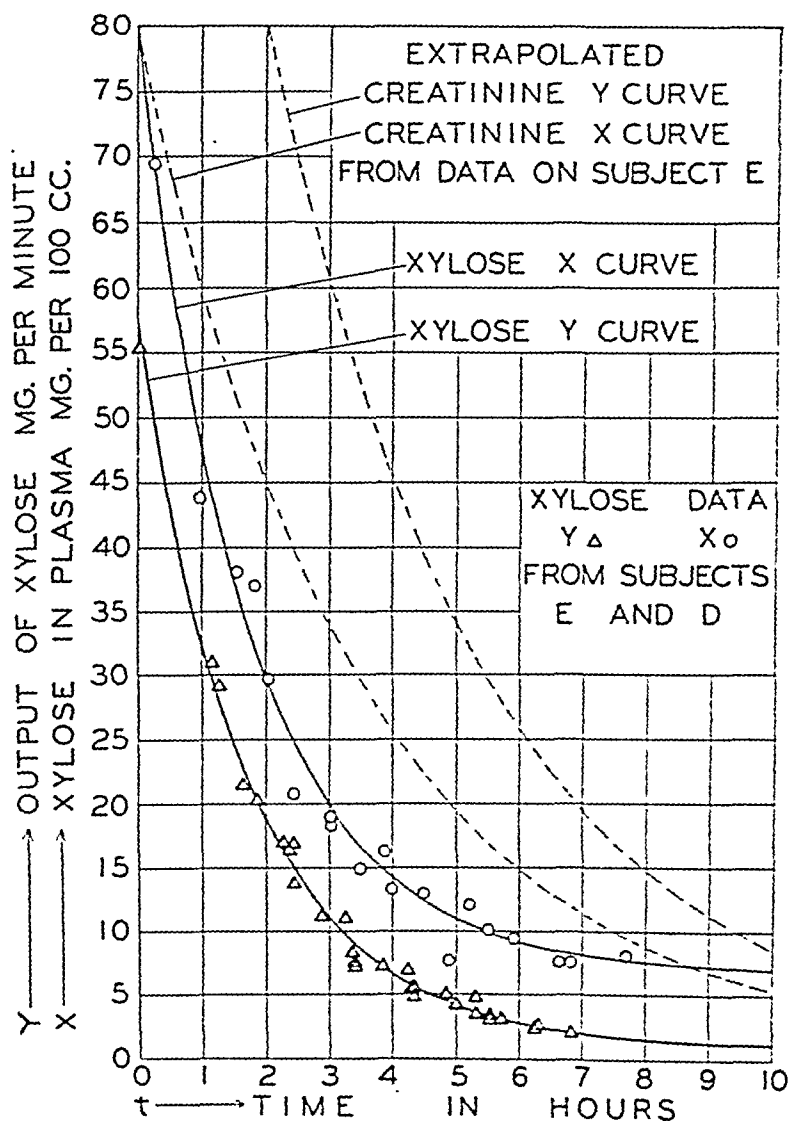
FIG. 1. TIME CHANGE IN PLASMA CONCENTRATION AND RATE OF OUTPUT OF XYLOSE IN MAN

The equation of the plasma curve (*x* curve) is $x = 6.87 + 72.65 \, e^{-0.526 \, t}$, that of the rate of output (*y* curve) is $y = 1.06 + 55.94 \, e^{-0.526 \, t}$. The observations of plasma concentration are represented by circles, those of rate of output by triangles. The corresponding curves of the data on creatinine (broken lines) have been drawn by extrapolating from the published equations (1) so that the plasma concentration of both substances begins at the same level. The asymptotes have not been drawn in order to avoid confusion. The larger scattering of the circles about the *x*-curve should be noted. The time given has no reference to the time of ingestion of the substance.

the rate of excretion is exponential, the mean rate of an interval does not lie theoretically on the midpoint of the interval. These two sources of error should be added to the ones already discussed (1) as influencing the final value of the excretion A.

Figure 1 shows the mean curves for the time change of the plasma concentration and the rate of output of xylose. The two corresponding curves for creatinine (extrapolated from the published equations (1)) have been drawn to enable comparison. In order to avoid confusion, the asymptotes have not been drawn. The larger scattering of the observations (represented in the figure by circles) about the curve of plasma concentration is evident. The difference in the endogenous output of reducing substances between the two subjects would have been imperceptible in the figure, and consequently no attempt was made to distinguish the points in the original drawing.

DISCUSSION

The method of analyzing the changes in plasma concentration and rate of output of a substance with respect to time, although incomplete, in that the initial phase following ingestion has not been considered, supplies a number of useful quantitative relations to be discussed in future publications. Here only the linearity of the resultant equation will be considered.

Equation 3 can be written

$$y = p + A x, \tag{4}$$

where

$$p = y_c - A x_c. \tag{5}$$

Substituting in Equation 4 the numerical values of the constants we obtain

$$\text{Subject E} \quad y = -4.0705 + 0.77 x, \tag{6}$$

$$\text{Subject D} \quad y = -4.2304 + 0.77 x. \tag{7}$$

The same situation is found here, therefore, that was found in the analysis of the creatinine data (1), namely, that the straight line representing the relation between the rate of output of xylose and the plasma concentration does not go through the origin of the coordinate system. Consequently the ratio (y/x), called " glomerular clearance " by Shannon, Jolliffe and Smith (8) is affected not only by the errors in y and x (and the larger errors are in the denominator), but also by a systematic error arising from the fact that p (Equation 4) is not zero. The ratio, from Equation 4, is

$$(y/x) = A + (p/x) \tag{8}$$

and is evidently a function of the plasma concentration. This systematic

error becomes larger the smaller the plasma concentration, but it is not due to errors in the latter.

In order to give a clear idea of the extent of the variation in the ratio from all the sources mentioned, it will be enough to calculate the ratios (clearances) for the data of this report. For Subject E the mean of the ratios is 0.51, with a range of 0.25 to 0.72; for Subject D, mean 0.52, range 0.32 to 0.70. The mean of the ratios is, therefore, far below the excretion constant calculated from the same data, and the variation of the ratio is such as to discourage any use of it. Of course, having assigned to this ratio a specific meaning, that of measuring the glomerular filtrate, the authors above mentioned regard this variation as quite in keeping with accepted views on the variability of the glomerular filtrate (see "Discussion" in (8)).

If, instead, a straight line is fitted to the very points the ratios of whose coordinates show such variation, the two equations result:

$$y = -3.258 + 0.729\,x, \tag{9}$$

$$y = -3.427 + 0.736\,x, \tag{10}$$

according as x or y, respectively, is assumed free from error. It is obvious then that the fitting of a straight line to data roughly interpolated *during their exponential decrease* would succeed, where the ratios had failed, in giving a close approximation to the constant calculated by the general method (Equations 6 and 7).

From Equation 8 it is also clear that the larger the plasma concentration the closer will the ratio approximate to the value of the excretion constant, the degree of approximation depending also on p. Hence, papers in which the ratios only are published are of no assistance in the calculation of the excretion constant. For this reason we are unable to analyze the data of Jolliffe and Chasis (9) on man. If the plasma concentration of their subjects was around 50 mgm. per 100 cc., as mentioned by Jolliffe and Chasis, their ratios, calculated from Equation 6 or 7, would be about 10 per cent below the value of the excretion constant. This will exaggerate the discrepancy between the excretion constant of our report (0.77) and the mean ratio of theirs (0.97 after correcting for the difference in units). Further comparison of their data with ours is rendered difficult by the circumstance that the points used for our calculations were taken from a different part of the curve. Our data were taken, as underscored above, during the exponential decrease of both curves, theirs apparently in the region of the maximum. It is not known whether identical relations hold for the whole curve, but even assuming such identity, the linear interpolation used to synchronize the data is unreliable in the region of the maximum. Whatever the case might be, we shall simply note that the excretion constant of Subjects E and D lies in the lower range of the ratios

recorded by Jolliffe and Chasis. The reader's attention is called to the fact that, in order to make this comparison, the published ratios of Jolliffe and Chasis should be multiplied by the surface area of the respective subject and corrected for units.

As a further illustration of the fallacy of the ratio, the recent report of Shannon (10) on the dogfish will be analyzed. From his summary and conclusions we shall quote the following:

"At plasma levels below 7 mgm. per cent the creatinine clearance appears to reach a maximum, varying in our experiments from 4.2 to 7.2, and averaging 5.8 times the xylose or sucrose clearance. As the plasma level is raised to higher values the creatinine clearance falls, approaching the xylose or sucrose clearance asymptotically."
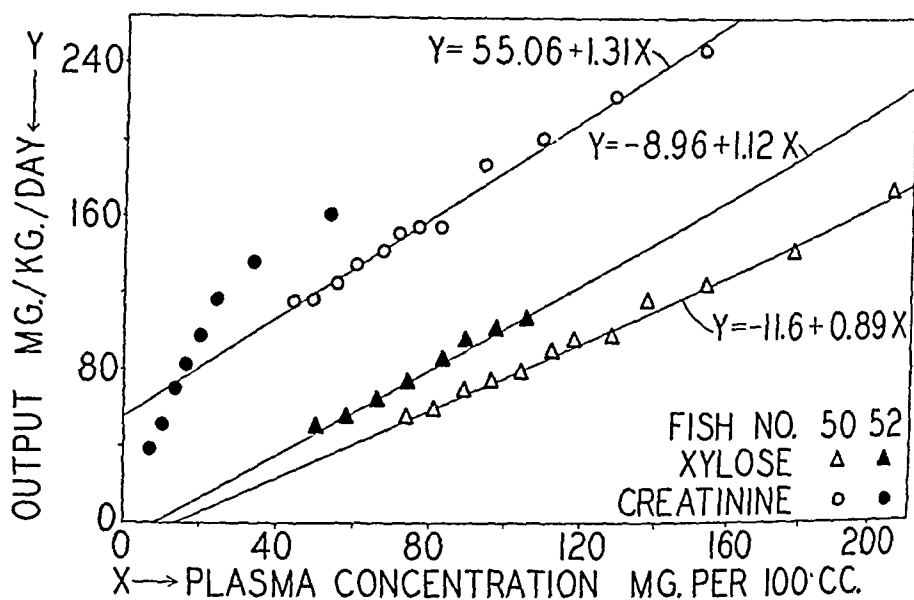
FIG. 2. STRAIGHT LINES FITTED TO THE DATA OF SHANNON (10) ON THE EXCRETION OF XYLOSE AND CREATININE IN THE DOGFISH

The observations on xylose are represented by triangles, those on creatinine by circles. The data from one fish (Number 50) are indicated by open symbols, those on the other (Number 52) by closed symbols. The points fall along straight lines except those of creatinine in Fish 52. Note the large intercepts of the three lines drawn, especially that corresponding to the creatinine data on Fish 50, giving rise to a systematic variation in the "glomerular clearance," ratio (y/x).

Since only two protocols were published, from two different animals, and no data were given in regard to the endogenous blanks, the general method of computation cannot be applied. But we can calculate the outputs from the published ratios (Table II (10)) and pair them with the (interpolated) plasma concentrations as given. By plotting on rectangular coordinates the following figure is obtained (Fig. 2). It is at once seen that the points of xylose fall along straight lines cutting the x-axis to the

right of the origin, as in the human case for xylose and creatinine, whereas the creatinine points show a remarkable behavior. Those of Fish 50 fall on a straight line intercepting the y-axis at $y = 55.0$ mgm. per kilo per day, but those of Fish 52 do not follow a straight line at all. Whether this is the normal behavior of the renal excretion of creatinine at low plasma levels (say below 25 mgm. per 100 cc.) in the dogfish, is not clear from the data, but whether it is so or not it is quite clear that the ratio (y/x) cannot be used. Leaving the creatinine data of Fish 52 out of consideration, the other three lines can be calculated as shown in the figure (see Fig. 2). Calculating the ratios for these equations, we get

Fish 50, creatinine, $(y/x) = 1.31 + (55.06/x)$,   (11)

Fish 50, xylose, $(y/x) = 0.89 - (11.60/x)$.   (12)

Consequently, the creatinine ratio will increase as the plasma creatinine decreases, and the xylose ratio will decrease as the plasma xylose decreases. Since the quantity p is larger for creatinine than for xylose, and the plasma concentrations are in general larger for xylose than for creatinine, it follows that the effect of a changing plasma concentration will be more pronounced with creatinine than with xylose. All these conclusions can be verified by examining Table II of Shannon's report (10) or by direct calculation from Equations 11 and 12.

Furthermore, if the ratio of the ratios is considered, as has usually been done, and one is careful not to use small values of plasma xylose which make the double ratio grow to infinity and then become negative, it will be seen, from Equations 11 and 12, that, for increasingly large values of plasma creatinine and plasma xylose, the double ratio will behave roughly as a decreasing function of the plasma creatinine alone. This behavior deceived Shannon as to the character (he calls it logarithmic) of the function. The function is algebraic, and, for large constant values of plasma xylose, its asymptote is not 1, as stated in the last sentence of the above quotation, but 1.47, equal to the ratio of the two excretion constants.

The theoretical considerations of Shannon, based, as they are, on a fictitious variation, are, of course, meaningless, but his experimental data should be carefully reexamined, because the numerous though erratic observations in the dog (11) suggests a close numerical value for the ratio of the two excretion constants in these two widely separated animal species (1.37 for the dog, 1.47 for the dogfish).

It would take an unnecessary amount of space to analyze the data on the dog, for which reason we shall limit ourselves to a group of data on one dog (11). Twenty-three observations are available, 12 from a long experiment, and 11 from three short experiments. By treating the latter

as if supplied by one experiment, and calculating the straight lines, we get

Dog 36, creatinine (long experiment) $y = 0.616 + 0.659\,x$, (13)

Dog 36, creatinine (short experiment) $y = 0.161 + 0.569\,x$, (14)

Dog 36, xylose   (long experiment) $y = 0.377 + 0.524\,x$, (15)

Dog 36, xylose   (short experiment) $y = 3.395 + 0.384\,x$. (16)

Although the creatinine data were obtained by two different methods of analysis, and moreover the plasma concentration of creatinine was in general much less than that of xylose, the data on xylose show by far the larger scattering and the coefficients of x (excretion constants) the greater discrepancy, confirming not only the relative inaccuracy of the determination of xylose concentration, but also our general conclusion that the variation in the ratio is due to errors of analysis, chemical or otherwise. Here too, we see that the constant term of the equations (Equations 13 to 16) will introduce a systematic variation in the ratio, but this term being small in magnitude (with the exception of that in Equation 16), the effect will be appreciable only at low plasma levels, and consequently will be easily detected in the creatinine ratios, but not at all evident in the xylose ratios. If we separate the ratios as given in the table (Table I (11)) according as the plasma concentration of creatinine is larger than 25 mgm. per 100 cc. or less, we get, for the mean of the ratios in the first group (10 observations), 79.3, and for the mean of the ratios in the second group (13 observations), 99.9. The authors of the paper quoted, naturally, attach no significance to this variation, because in their experience the ratios of the dog vary " between 40 and 150 per sq. m.," but, in view of the fact that the variation in the ratio includes all the variations that can occur in the kind of experiments usually made, it will be immediately seen that the excretion constant cannot exhibit such disconcerting variation, even if uncorrected for the surface area or the weight of the animal.

Pending a better determination of the constants we can say that the excretion constant of creatinine in the dog is 1.26 or 1.48 times that of xylose, according as we take Equations 13 and 15, or Equations 14 and 16. All the double ratios (Table I (11), column 10) fall within these limits.

In man, as far as the observations on our Subject E are concerned, the excretion constant of creatinine is 2.36 times as large as that of xylose, and is, therefore, in the upper range of the double ratios calculated by Jolliffe and Chasis (9). Since in calculating the ratio of two simultaneous " clearances " the urine flow cancels out, the double ratio is nothing more than the ratio of two concentration indices. For the double ratio to approximate, within its wide variation limits, to the ratio of two excretion constants it is necessary and sufficient that the excretion constants be independent of urine flow. That the excretion of xylose is independent of

urine flow is shown by examining the large fluctuation of urine flow in our experiments (Table I) or the mean diureses in Table II, especially those of Subject E, and noticing that such fluctuations have no effect either on the regularity of the curves or on the distribution of the errors. That the excretion of creatinine is also independent of urine flow has been shown previously (1).

In regard to the physiological significance of the excretion constant, even if considered as the corrected value of a "clearance," we can say, supplementing what was said a propos of creatinine, that the exponential law is one of the few fundamental relations between two magnitudes and is shared by a multitude of physical and chemical phenomena. It would be unjustified, therefore, from the exponential decrease of plasma concentration and rate of output, or from its consequence, the linear relationship between these two quantities, to draw any conclusions about the intricate mechanism whereby a substance is removed from the plasma.

A large number of inert substances can be imagined to be excreted by the kidney in a manner similar to that of creatinine or xylose, which may give a corresponding series of constants. And, if these constants could be arranged according to their magnitude in the same order as that of some physical property of the substances, their diffusion constants, for instance, the information so derived might lead to a better understanding of the inner working of the kidney.

Finally, it should be pointed out, that, from the superficial agreement between the variability of the "clearance" and the variability of the glomerular filtration rate, it cannot be argued that the relative stability of the excretion constant is incompatible with its representing the filtration rate, because the instability of every one of the elements of an assemblage does not preclude the stability of the whole, witness body temperature, diastolic blood pressure, etc.

SUMMARY AND CONCLUSIONS

The excretion of xylose has been investigated by a method previously used, which determines the time change of plasma concentration and rate of output of the substance during their phase of exponential decrease. The method establishes the linearity of the relation between these two quantities, supplies the necessary constants, and besides gives explicit information on the errors of observation. The excretion constant of xylose, calculated from data on two human subjects, is 0.77, when the plasma concentration (x) is measured in mgm. per 100 cc. and the rate of output (y) in mgm. per minute. This constant is independent of moderate exercise and moderate changes in diuresis.

The behavior of the two quantities (y and x) with respect to time is illustrated graphically and the corresponding curves of creatinine, previously obtained, are also included to enable comparison.

Other published data on man are either too incomplete to be analyzed by this method, or are given only in the form of a ratio (y/x).

It is shown that the ratio of the two quantities (y/x) is disturbed not only by accidental errors of observation (and the larger errors are in the denominator), but also systematically by the fact that the straight line satisfying the relation between y and x does not go through the origin of the coordinate system. This systematic variation in the ratio is found to be relatively unimportant in the case of the dog, but quite serious in the published data on the dogfish. The danger of interpreting variations in the ratio in terms of renal physiology is made obvious.

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THE CHARACTERISTICS OF SYNOVIAL FLUID IN GONOCOCCAL ARTHRITIS

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To anyone who has studied a group of cases of gonococcal arthritis, it is plain that some patients recover entirely without any permanent disability. In a large number, however, the disease is progressive and results in a chronic arthritis with resulting restriction of motion or ankylosis. The reasons for the variations observed are not clear in every case, although the type and severity of the reaction in the joints are of the highest importance. Keefer, Parker and Myers (1) have shown that the pathologic process, as determined by the histological examination of the joints of patients with gonococcal arthritis, varies tremendously in its severity and extent. When there is pain, periarticular swelling and slight exudation of fluid into the joint cavity, the process is confined for the most part to the subsynovial connective tissue where there are collections of polymorphonuclear leukocytes, lymphocytes and plasma cells, about the blood vessels and between the strands of connective tissue. The surface synovial cells are intact. In other cases in which the synovial fluid is excessive and contains many cells, there develops a much more extensive inflammatory reaction of the synovial membrane and underlying connective tissue. In such cases the superficial synovial cells are entirely destroyed leaving only a layer of granulation tissue with newly formed blood vessels, many polymorphonuclear leukocytes and numerous micro-organisms. The deeper parts of the synovial membrane are not extensively involved. In such instances, there may be destruction of the cartilage and underlying bone with a resulting fibrous or bony ankylosis.

In view of the varied pathologic picture and the uncertainty of the outcome in a given case, we studied the synovial fluid from forty cases of gonococcal arthritis in an effort to gather more exact information regarding the alterations that occur and to determine if any correlations could be made between the type of reaction and the subsequent outcome of the joint disorder.

METHODS

Fluid was withdrawn from the affected joints with a needle and syringe, using the usual aseptic precautions. The fluid was obtained from the knee joints

in thirty-seven cases and from the wrist, ankle, and olecranon bursae respectively in the remaining three. In all, seventy-seven specimens from forty patients were studied. The amounts removed varied from three to 250 cc. with each aspiration. The total and differential cell counts were made immediately. Most of the differential counts were done using the supravital method described by Forkner. A few were made following the staining of a smear with Wright's stain. The former method proved to be the more satisfactory. All specimens were cultured for gonococci and smears of the exudate were stained, according to the Gram technique, for organisms. Chemical examinations were made on oxalated fluid, and included total protein, nonprotein nitrogen and sugar. In some cases the results of the determinations were compared with those for the nonprotein nitrogen and sugar in the blood at the time the joints were aspirated. Gonococcal complement fixation tests were done upon both the blood sera and the synovial fluids.

The diagnosis of gonococcal arthritis was made from: 1. the history of a recent attack of gonorrhea; 2. the presence of a localized gonococcal infection as proven by symptoms, signs and bacteriologic examination; 3. the presence of gonococci in the synovial fluid or a positive gonococcal complement fixation test in the blood serum or synovial fluid. In the cases in which gonococci were not demonstrated in the synovial fluid, care was taken to exclude other types of arthritis, including rheumatic fever and tuberculous arthritis. In no case, however, was the diagnosis of gonococcal arthritis accepted without *at least* finding a localized gonococcal infection or a positive gonococcal complement fixation test in the blood or synovial fluid.

General characteristics of fluid

The fluid varied in color and clarity from pale yellow to yellow and from slight turbidity to a definite cloudiness. It clotted upon standing, but in many cases the clot was not complete for a period of several days. Mucin was frequently abundant and it was found that unless the fluids were studied immediately, the presence of mucin interfered with the examinations.

From the cultures of sixteen fluids from ten patients gonococci were readily grown in pure culture. These organisms were identified by their morphological and fermentation characteristics and, in some instances, by agglutination to known antisera. No organisms were grown from the remaining fluids.

It was surprising that we were able to cultivate the gonococcus from the synovial fluid in only twenty-five per cent of the cases. It might be said that we were unable to cultivate them from the remaining fluids due to a lack of the proper technique, or that the gonococci were present in such small numbers that they were not detectable upon artificial cultivation. We have, however, discarded both of these objections, inasmuch as opportunities were present for two different laboratories to examine the fluid for micro-organisms; one the Bacteriological Laboratory of the Boston City Hospital, and the other our own; and in but one exception the results of cultivation were the same. It was, in addition, possible to cultivate gono-

cocci whenever they were found in stained smears made from the fluid. On the other hand, we were occasionally able to cultivate organisms from synovial fluid when we were unable to find them in stained smears. From the results of our pathologic studies of two cases, we have come to another conclusion regarding our failure to grow organisms from the synovial fluid in every case; namely, that the inflammatory reaction in the cases with non-infected fluids is chiefly below the surface of the synovial membrane, and in the periarticular tissues. In such cases, the surface layer of synovial cells is intact and no organisms can be cultivated from the fluid, but they may be present in the synovial tissues beneath the surface where they may be found on microscopic examination of the tissue.

In the cases in which organisms can be cultured with ease, the surface of the synovial membrane is destroyed, and is the site of an intense inflammatory reaction. In other words, in the cases with infected fluids the organisms have extended from the periarticular tissues into the synovial cavities and destroyed the synovial lining of the joint; in those with non-infected fluids, the inflammation is confined to the periarticular tissues beneath the surface of the synovial membrane.

This explanation is not far-fetched when one recalls the reaction in other serous membranes when infection is present in the neighborhood, for example, the sterile pleural effusions in pneumonia, the aseptic meningitis in extra-dural or brain abscess or the sterile effusions in the joint cavities in the presence of osteomyelitis.

The total cell counts of the fluids varied between 1,800 and 158,000 per cubic millimeter, varying from 7,350 to 158,000 per cubic millimeter for infected fluids and for non-infected fluids from 1,800 to 78,250 per cubic millimeter. While the variations in both groups were wide, it was true that higher cell counts were found more often in the infected than in the non-infected synovial fluids.

In all of the fluids, the polymorphonuclear leukocytes predominated and fluctuated so as to form from forty-six to 100 per cent of the cells. The other common cells were clasmatocytes and monocytes. Of the former, there were between one and sixteen per cent, and of the latter between one and thirty-three per cent. Only rarely were the lymphocytes increased above ten per cent, although the extreme variations were between one and thirty-one per cent. In the infected fluids polymorphonuclear cells were always over seventy-six per cent. The non-infected fluids more often had higher monocyte and clasmatocyte counts than infected fluids, especially when the total count was low. On the whole, then, the non-infected fluids showed a somewhat lower total cell count, and contained more monocytes and clasmatocytes than the infected fluids. The total and differential cell counts are recorded in Table I.

These findings are in agreement with the cellular reactions in other serous sacs when the fluid is infected or non-infected. Thus Scott and

Summary of total and differential cell counts of synovial fluids in gonococcal arthritis

Number of fluids	Total cells per cubic millimeter	Polymorphonu-clear cells	Lympho-cytes	Mono-cytes	Clasmato-cytes	Eosino-phils
	thousands	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
		Infected fluids				
2	7.3–10.0	76–85	6–12	5–7	2–5	0
4	10.1–40.0	86–99	3–5	1–4	0–12	1–2
5	40.1–60.0	89–98	1–6	1–4	1–3	0
3	60.1–158.0	92–99	1–6	0–2	1–2	0
		Non-infected fluids				
20	1.8–10.0	46–100	1–31	1–33	1–19	0-2
28	10.1–40.0	82–99	1–8	1–7	1–6	
4	40.1–60.0	93–99	1–4	1–5	1–4	0-2
1	60.1–78.2	87–99	1–4	1–3	1–6	0

Finland (2) found that when the pleural fluid in pneumonia was non-infected the number of clasmatocytes, monocytes and lymphocytes was much higher than in the infected fluids. This was especially true when the effusion remained sterile. If the fluid became infected, practically all the cells were polymorphonuclears. The same type of reactions may be observed in the cerebrospinal fluid during the course of meningitis, resulting from an extra-dural abscess. The differential cell count, then provides some information regarding the presence or absence of organisms in synovial fluid in gonococcal arthritis.

Gonococcal complement fixation test

This test was done forty-eight times on both the blood serum and synovial fluid from twenty-seven cases. The reaction was positive in thirty-one synovial fluids, doubtful in one, and negative in sixteen. Stating it in another manner, it was positive in seventy-one per cent of the cases of gonococcal arthritis when the synovial fluids were studied. When the results of the test in the blood sera and the synovial fluids were compared, there was disagreement in only six instances. The reaction of the synovial fluid was negative on four occasions when that of the blood was doubtful or positive. The gonococcal complement fixation was doubtful once with synovial fluid when the blood serum was negative, and the synovial fluid was positive in one instance in association with a doubtful blood serum reaction. In three cases both the blood serum and synovial fluid were negative early in the course of the disease when gonococci were

present in the synovial fluid.   In three other cases, it was possible to observe the change of the reaction in the blood serum and synovial fluid from negative to positive.   The blood serum always showed a positive gonococcal complement fixation reaction before the synovial fluid.

In another communication (3) we have analyzed in greater detail the results of the gonococcal complement fixation test in the blood sera and synovial fluids from cases of gonococcal arthritis as well as other types of arthritis.   It may be repeated here that this test has been found to be of great value to us in the etiological diagnosis of gonococcal arthritis.

Total proteins in synovial fluid

These were determined in samples of fifty-four synovial fluids and varied between 3.6 and 6.0 grams per cent.   This value included the nitrogen of the mucin, inasmuch as it was not removed by precipitation before the Kjeldahl determinations were made.   The amount of protein in the infected and non-infected fluids was essentially the same, although all of the infected fluids had a protein content of five grams per cent or more. There was no correlation between the amount of total protein and the non-protein nitrogen, nor was there any relationship between the total protein and the total number of cells.

Nonprotein nitrogen in blood and synovial fluid

The nonprotein nitrogen content of both the blood and synovial fluid was determined simultaneously in thirty-seven instances.   In the synovial fluid it varied between fifteen and forty milligrams per cent.   In fourteen cases, the nonprotein nitrogen of the blood was somewhat higher than that in the synovial fluid, in seventeen others the values were the same or varied within a limit of two milligrams, and in four samples the synovial fluid contained slightly more nonprotein nitrogen than the blood.   The differences between the two were never very great.   Moreover, there was no evidence that the presence of bacteria in the synovial fluid increased the quantity of nonprotein nitrogen, and there was no correlation between the number of cells and the amount of nonprotein nitrogen.

From a comparison of the values in both the blood and synovial fluid, it appeared that the amount of nonprotein nitrogen in the latter was dependent upon the total amount in the blood, the differences between the two were never very striking and, in some cases, the values were precisely the same.

Sugar content of the synovial fluid

The sugar content of the synovial fluid varied between 38 and 131 milligrams per cent.   In most cases it was lower in the synovial fluid than in the blood, and this was especially noticeable in the infected fluids.   Seldom was the sugar content of the synovial fluid higher than that of the blood.

That this did occur can be explained by the fact that the joints were not aspirated after the patients had been fasted, though they were frequently collected several hours after a meal. Thus, the higher content of sugar in the synovial fluid can probably be explained upon the basis of a higher blood sugar sometime before the joint was aspirated; that is to say, the sugar content of the joint fluid remained high for a longer time than the sugar of the blood. Similar observations have been recorded by Cajorie, Crouter and Pemberton (4), Allison, Fremont-Smith, Dailey and Kennard (5). It was true then, that the level of the synovial fluid sugar depended to some extent upon the amount of sugar in the blood at the time the aspiration was performed, or the amount present in the blood several hours previously. There were, in addition, two other factors of significance; namely, *the presence of organisms and the number of cells.* The higher the total cell count in the synovial fluid the lower the sugar; and when there were organisms present in the fluid, even if the total cell count were low, the sugar content was reduced. This is shown in Figure 1.
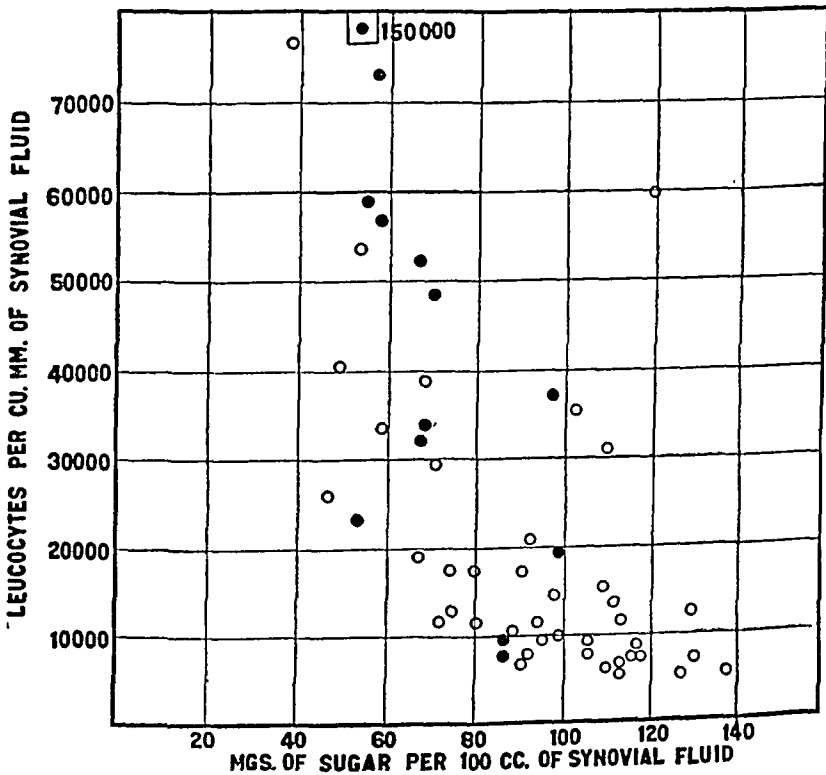
FIG. 1. CHART SHOWING VARIATION IN THE SUGAR CONTENT OF THE SYNOVIAL FLUID AND THE TOTAL LEUKOCYTE COUNT
The solid dots indicate infected fluids and the circles, non-infected fluids.

The results of the determinations of the total proteins of the synovial fluids together with the nonprotein nitrogen and sugar content of both the blood plasma and synovial fluids are summarized in Table II.

TABLE II

Summary of the non-protein nitrogen and sugar content of blood and synovial fluid in twenty cases

Num-ber of fluids	Total protein joint fluid	Blood plasma nonprotein nitrogen	Synovial fluid nonprotein nitrogen	Blood sugar	Synovial fluid sugar
	grams per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent
Infected fluids					
10	5.4–5.9	17–34	20–37	82–108	52–86
Non-infected fluids					
25	3.8–6.0	19–41	21–33	82–175	52–131

Briefly, then, the sugar content of the synovial fluid depended upon three factors: the level in the blood, the number of leukocytes and the presence of organisms.

Relation between type of reaction in the synovial fluid and the outcome of the arthritis

In view of the fact that gonococcal infections of the joints frequently lead to a crippling disease and, in some cases, to death, we were interested in analyzing the outcome of the patient's illness in the light of the various characteristics of the synovial fluid. In a number of cases, this is a task of no small difficulty in view of the chronic nature of the pathological process and the relapses that occur, especially following a reinfection of the genital tract. We have records of the outcome of thirty-three cases. The results are summarized in Table III.

TABLE III

The outcome of thirty-three cases of gonococcal arthritis

	Patients with infected synovial fluid	Patients with non-infected synovial fluid	Per cent
Died..........................	2	0	6
Well..........................	2	6	25
Chronic joint disease.............	5	18	69

From this table, it is obvious that gonococcal arthritis is a serious disease, whether the synovial fluid is infected or non-infected at the time of the examination. Thus, sixty-nine per cent of the patients had some evidence of chronic changes in their joints following the infection. This disability varied from slight impairment of function to considerable limita-

tion of motion. In order to gain further information we studied the cell count of the synovial fluid of the patients in the different groups to determine whether this response gave any indication of the possible outcome in a given case. It was found that in no instance was recovery complete when the synovial fluid cell count was above 40,000 per cubic millimeter. This was true whether the fluid was infected or non-infected. On the other hand, of the patients with synovial fluid cell counts below 40,000 only thirty-seven per cent recovered completely. In other words, from this small series of cases, a synovial fluid cell count over 40,000 was invariably followed by some permanent change in the joints, and complete recovery occurred in only thirty-seven per cent of the cases with counts below 40,000 per cubic millimeter. These facts would seem to indicate that the cell count of the synovial fluid may serve as a very crude index in determining the outcome in a given case. There is another factor of some importance, namely, the presence of organisms in the fluid. Complete recovery was observed in only two of the nine patients with infected fluids. Briefly, it may be said that the outlook is not good as far as complete recovery is concerned if the joint fluid is infected and the leukocyte count high; on the other hand, the better results were seen in patients with low cell counts and non-infected fluids. The results are tabulated in Table IV.

TABLE IV

The outcome of thirty-three cases of gonococcal arthritis with cell counts in synovial fluid above and below 40,000 per cubic millimeter

	Infected fluids	Non-infected fluids	Died	Well	Chronic joint disease
Patients with cell counts in synovial fluid over 40,000 per cubic millimeter.	7	4	2 0	0 0	5 4
Patients with cell counts in synovial fluid under 40,000 per cubic millimeter.	2	20	0 0	2 6	0 14

DISCUSSION

From the data presented, we may now return to the two questions we set out to answer. First, what information of diagnostic value can be obtained from the examination of the synovial fluid in a suspected case of gonococcal arthritis, and, second, does the character of the joint fluid provide any information regarding the prognosis in a given case?

It will be universally agreed that the most important aid in establishing a diagnosis of gonococcal arthritis is the demonstration of gonococci in the synovial fluid. However, in most of the cases we studied it was not possible to cultivate organisms from the synovial fluid or stain them in

the exudate at the time it was examined. In these, the diagnosis, as far as the joints are concerned, must be based upon indirect evidence. The chemical examinations of the fluid revealed no information of specific diagnostic value. The nonprotein nitrogen of the joint fluid was the same as that of the blood. The amount of sugar in the synovial fluid depended upon three factors; namely, the level of the sugar in the blood, the number of leukocytes and the presence of micro-organisms. Inasmuch as high leukocyte counts and low synovial fluid sugar content were found in noninfected fluids, the presence of a low sugar content did not indicate the presence of organisms in every case. The total protein and the total and differential cell count were of significance insofar as they indicated the presence of an exudate, but the wide variations in the number and types of cells, in both the infected and non-infected fluids, forced one to the conclusion that these findings in a given case were of little value in discriminating the infected from the non-infected fluids. The gonococcal complement fixation test with the synovial fluid, as well as with the blood serum was of distinct value and, since it was found to be positive in a large percentage of cases and highly specific, it proved of considerable diagnostic aid.

From the diagnostic point of view the most important examinations were the bacteriological, the cytological, and the serological tests. The chemical examination yielded very little significant information.

From the point of view of prognosis, the characteristics of the fluid were of some importance. While it was found that the prognosis was poor in the group as a whole, as far as complete recovery was concerned, the cases with the poorest outlook were those with high synovial fluid cell counts and infected fluids. As a rule, the patients who recovered completely were those with non-infected fluids and low leukocyte counts, or those with infected fluids and low leukocyte counts.

SUMMARY AND CONCLUSIONS

The synovial fluids from forty cases of gonococcal arthritis were studied to determine: (1) the various biological and chemical characteristics of the fluid and (2) whether or not information of value in diagnosis and prognosis could be discovered. The following results were obtained.

1. When the joints became involved as a result of a gonococcal infection, the synovial fluid was either infected or non-infected. In either case, the fluid had the characteristics of an exudate as judged by both the total protein and cell content.

2. The total synovial fluid cell count was increased in both types of fluid but, as a rule, it was somewhat higher in the infected fluids. There were, however, wide variations.

3. The differential cell count was of greater importance than the total cell count in the two groups of cases. In practically all, the polymorpho-

nuclear cells predominated. In the non-infected fluids, the clasmatocytes, monocytes and lymphocytes were present in much larger numbers than in the infected fluids.

4. The nonprotein nitrogen content of the synovial fluid was the same as that of the blood regardless of the presence of organisms or of a high cell count.

5. The sugar content of the synovial fluid varied with the level of the blood sugar, the number of leukocytes and the presence of bacteria. Of these factors the first two were of greater importance than the third.

6. The results of gonococcal complement fixation tests on the synovial fluid and blood were in agreement.

7. The bacteriological, cytological and serological tests were of the greatest value in providing information of diagnostic value.

8. The chemical examination of the fluid revealed no information of diagnostic importance.

9. While the prognosis, as far as complete recovery was concerned, was poor, the presence of micro-organisms and a high leukocyte count were more often followed by chronic joint disease than when there was a low leukocyte count and a sterile fluid.

We acknowledge our thanks to Miss Marjorie Jewell and Miss Eleanor Fleming for technical assistance.

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THE URINARY EXCRETION OF IODINE. I. THE LOSS OF IODINE IN THE URINE FOLLOWING THYROIDECTOMY

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INTRODUCTION

The purpose of this paper is to show that immediately following thyroidectomy for goiter, there ensues a great increase in the urinary excretion of iodine. This may amount to so much as 2.0 mgm. during a single twenty-four hour period. Since we have found (1) (2) (3) that the usual urinary excretion of iodine, of our hospital patients, ranges from 0.025 to 0.075 mgm. daily, this amounts to a considerable postoperative loss. Subsequently, after periods of varying length, the excretion of iodine in the urine returns to normal.

In investigating iodine metabolism, in patients with goiter (2) (4) (5) we have made routine determinations of the blood and urinary iodine (6). These have been correlated with the clinical findings (4) (5), and, particularly, with the results of the different forms of therapy employed. The data here presented were obtained during the progress of that study.

LITERATURE

Alexander Sturm (7) determined the urinary excretion of iodine, in dogs, subsequent to total extirpation of the thyroid. He observed a small, but definite, increase in the urinary output of iodine following the thyroidectomy. Curtis and Barron (8) reported a striking postoperative increase in the urinary excretion of iodine, in four patients, subsequent to total thyroidectomy for hypertensive cardiovascular disease in man.

In reporting certain clinical features of the urinary excretion of iodine, Curtis and Phillips (1) noted that the urinary excretion of iodine is greatly increased following partial thyroidectomy for goiter. They also observed an increased urinary loss of iodine after subtotal thyroidectomy for toxic goiter (4) (9). The usual daily loss of iodine, in the urine of normal individuals, is similar to that of University Hospital patients (3).

In reporting the increased urinary excretion of iodine, in patients with toxic goiter, Curtis and Phillips (9) stated that an increased postoperative iodine loss ensues in patients operated upon for causes other than goiter. This was observed in five instances by Curtis and Cole (10).

OBSERVATIONS

The pre and post-operative urinary excretion of iodine has been determined in five patients, all thyroidectomized for goiter. These were all maintained upon the Surgical Research Service, in the University Hospital. They were given the usual hospital diet, excluding only those foods known to have a high iodine content (11). Iodized salt is not used in our hospital diets. They were kept at bed rest, and no iodine, in any form, was used or administered throughout their entire management. Their only daily source of iodine was in the food, water, and air intake.

Blood samples, for the blood iodine determinations, were drawn mornings, in the postabsorptive state. Collection of the twenty-four hour urine specimens was made by trained attendants, and was carefully supervised. Analyses of the iodine content of the blood and urine were made after the method of Phillips and Curtis (6). This is an adaptation of the von Fellenberg procedure (14).

Protocols, presenting the principal findings, and the management of each patient, are given, together with corresponding tables and figures which show particularly the urinary excretion of iodine. The five patients each present a different form of goiter: (1) Non-toxic nodular goiter, (2) mildly toxic nodular goiter, (3) toxic nodular goiter, (4) diffuse colloid goiter and (5) exophthalmic goiter. In all five there ensued a definite increase in the urinary iodine loss after thyroidectomy.

Patient 1. Non-toxic nodular goiter (Figure 1)

B. N., No. 326858, a housewife aged 32, entered the University Hospital December 28, 1933, for thyroidectomy. She had noted a preadolescent goiter, which had slowly become more evident. There had been questionable mild toxic episodes. On December 31 her basal metabolic rate was plus 5, with the pulse 72, temperature 98.6, respirations 15 and blood pressure 105/72, in the basal state. There had been mechanical symptoms, and the trachea was found by x-ray to be deviated. The larynx was normal. The heart was negative.

Laboratory investigation revealed a negative urine, negative Wassermann and Kahn reactions, a secondary anemia and a normal differential count. The blood iodine was elevated, 19 gamma [1] per cent on December 30, as compared to a normal of 12 (12). The findings of the usual chemical analyses of blood were otherwise within the normal range.

She was prepared for thyroidectomy by rest alone. *No iodine was used or administered* throughout her management. The daily twenty-four hour urinary excretion of iodine was determined, Figure 1. During the six days preceding the thyroidectomy it remained unusually constant, ranging from 51 to 73 gamma (.051 to .073 mgm.), and averaging 64 gamma (.064 mgm.).

Thyroidectomy was accomplished on January 6, under nitrous oxide anesthesia. Fifty grams of nodular colloid goiter were removed, leaving a portion estimated as of about 12 grams. The goiter was composed largely of small

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[1] A gamma is 0.001 mgm. (one one-thousandth of a milligram). 19 gamma per cent would be 0.019 mgm. in 100 cc. of blood.

colloid nodules, with a moderate amount of internodular substance. There ensued a moderate reaction and an otherwise uneventful convalescence.

During the twenty-four hours subsequent to the thyroidectomy there ensued a great increase in the urinary excretion of iodine, Figure 1. This reached 1.366 mgm., or more than twenty times the normal amount. During the following four days it gradually subsided. However, it was greater than usual when the patient was dismissed on January 11, 1934. The average daily urinary excretion of iodine during the five postoperative days was 521 gamma (0.521 mgm.). The basal metabolic rate subsequently fell to minus 6. On January 11 the blood iodine was 16.9 gamma per cent.
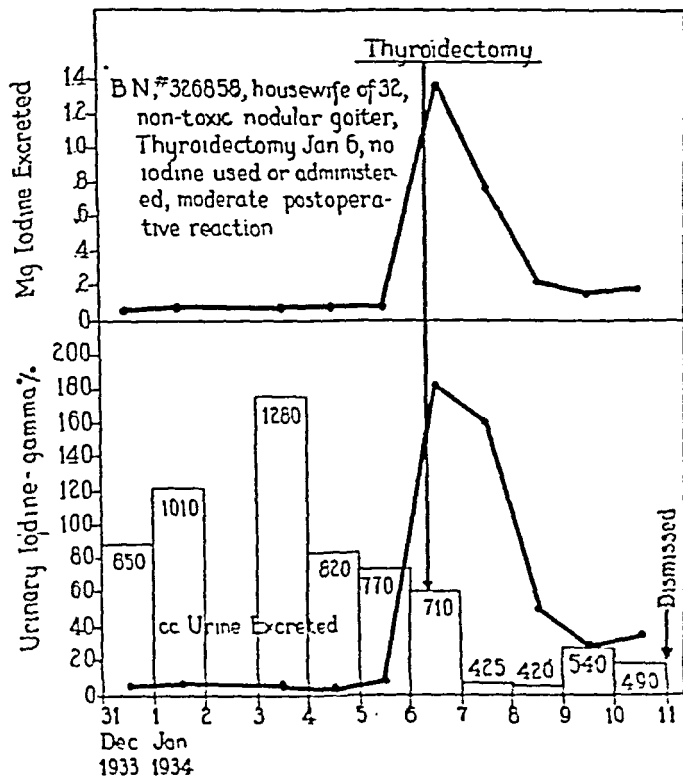
FIG. 1. THE URINARY EXCRETION OF IODINE IN NON-TOXIC NODULAR GOITER

Comment. The preoperative loss of iodine was unusually constant. It was within the normal range. A great increase followed thyroidectomy, and did not return to normal in five days. Following thyroidectomy there ensued a marked increase of the iodine concentration in the urine. The postoperative excretion of urine, however, was diminished.

Patient 2. Mildly toxic nodular goiter (Figure 2)

D. R., No. 330517, a young woman of 27, entered the University Hospital, February 2, for thyroidectomy. She had noted a goiter for seven years, and that

this had gradually increased in size. There had been definite toxic episodes. On February 6, 1934, after four days of bed rest, her basal metabolic rate was plus 18, with the pulse 92, the temperature 98.8° F., the respirations 17 and the blood pressure 130/80 during the basal state. There was some tracheal compression and also x-ray evidence of calcification in the goiter. The larynx was normal. The heart was negative, save for a sinus tachycardia.

FIG. 2. THE URINARY EXCRETION OF IODINE IN MILDLY TOXIC
NODULAR GOITER

Laboratory investigation revealed a negative urine, negative Wassermann and Kahn reactions, a moderate secondary anemia and, in the differential count, an increase in the monocytes. The blood iodine on February 6 was elevated, 15.4 gamma per cent, as compared to a normal of 12. The results of chemical examinations of the blood were otherwise within normal range.

She was prepared for thyroidectomy by bed rest alone, and *no iodine was used or administered* at any time during her management. The daily preoperative urinary excretion of iodine varied from 85 to 276 gamma (.085 to .276 mgm.), averaging 201 (.201 mgm.). There was an unusual and unexplained

decrease in the urinary iodine loss during the twenty-four hours preceding the thyroidectomy.

Thyroidectomy was accomplished on February 7, under nitrous oxide anesthesia. Ninety grams of nodular goiter were removed, leaving a portion estimated as of about 14 grams. The goiter consisted of colloid nodules of varying sizes with a considerable amount of intervening diffuse colloid substance. There was evidence of calcification, fibrosis, hemorrhage, and, microscopically, of "marked colloid involution." No cysts were found. There ensued a moderate reaction and, otherwise, an uneventful convalescence. On the second postoperative day the temperature rose to 103° F., and the pulse to 126.

During the 48 hours subsequent to the thyroidectomy there was a striking increase in the urinary excretion of iodine. This averaged 1.5 mgm. for the two immediately postoperative days. It then subsided, but was still elevated upon dismissal, February 12. The basal metabolic rate subsequently fell to minus 10. On February 11 the blood iodine was 13.9 gamma per cent.

Comment. The preoperative excretion of iodine was increased. There occurred a definite decrease just previous to thyroidectomy. The postoperative increase in iodine loss was marked. Subsequent to the first three postoperative days, the amount of urine and the iodine concentration were similar to the preoperative findings.

Patient 3.   Toxic nodular goiter (Figure 3)

C. C., No. 330699, a housewife of 31, entered the University Hospital, February 10, 1934, for thyroidectomy. Goiter had been originally noted at adolescence. It had become definitely larger during pregnancies. There had been evidence of toxicity for which she had been intermittently given Lugol's solution during the past three years. She had received no iodine for a month previous to this investigation. The basal metabolic rate on January 28 was plus 29, with the pulse 90, temperature 98.4° F., respirations 16 and blood pressure 142/78, in the basal state. The blood iodine on January 28 was increased, to 26.9 gamma per cent.

Laboratory investigation revealed a negative urine, negative Wassermann and Kahn reactions, and a normal blood picture. The heart was negative save for a sinus tachycardia. The trachea and larynx were negative. On February 15 the blood iodine was 21.2. The usual chemical analyses of blood gave normal values.

She was prepared for thyroidectomy by bed rest alone, and *no iodine was used or administered* throughout the entire management. The daily urinary excretion of iodine was determined. Figure 3. During the eight days preceding the thyroidectomy it ranged from 196 to 415 gamma (.196 to .415 mgm.), averaging 269 gamma (.269 mgm.) daily. Since the average normal daily urinary iodine loss in our hospital patients ranges from 25 to 75 gamma, the urinary excretion of iodine was greatly increased. The increased urinary iodine loss may have been a sequel of the previous administration of Lugol's solution. However, she had received no iodine for one month prior to operation. On February 21 the basal metabolic rate was plus 16, and on February 20 the blood iodine was 11.8 gamma per cent.

Thyroidectomy was performed on February 23, under nitrous oxide anesthesia. Eighty-four grams of nodular goiter were removed, leaving a portion estimated as of about 18 grams. The goiter consisted of many colloid nodules.

this had gradually increased in size. There had been definite toxic episodes. On February 6, 1934, after four days of bed rest, her basal metabolic rate was plus 18, with the pulse 92, the temperature 98.8° F., the respirations 17 and the blood pressure 130/80 during the basal state. There was some tracheal compression and also x-ray evidence of calcification in the goiter. The larynx was normal. The heart was negative, save for a sinus tachycardia.

FIG. 2. THE URINARY EXCRETION OF IODINE IN MILDLY TOXIC NODULAR GOITER

Laboratory investigation revealed a negative urine, negative Wassermann and Kahn reactions, a moderate secondary anemia and, in the differential count, an increase in the monocytes. The blood iodine on February 6 was elevated, 15.4 gamma per cent, as compared to a normal of 12. The results of chemical examinations of the blood were otherwise within normal range.

She was prepared for thyroidectomy by bed rest alone, and *no iodine was used or administered* at any time during her management. The daily preoperative urinary excretion of iodine varied *from 85 to 276 gamma* (.085 to .276 mgm.), averaging 201 (.201 mgm.). There was an unusual and unexplained

without nodules, and without thrill or bruit. Goiter had been noted since child-hood. It became more evident at puberty. During the past years had occurred a "nervous breakdown," and other episodes suggestive of toxicity. Her symp-toms were aggravated during menstruation and after severe emotional excite-ment. Tachycardia had been noted. Her mother had a goiter. On March 9, after bed rest, her basal metabolic rate was plus 10 (Table I). There were no pressure symptoms, and the trachea was in normal position by x-ray. The larynx and heart were found to be normal.

TABLE I

Twenty-four hour urine iodine in Patient 4
(M. S., No. 330981, housewife of 29, diffuse colloid goiter, no iodine used or administered, no postoperative reaction, uneventful convalescence)

| Date* | Vol-ume | Iodine gamma | Iodine excreted | Blood iodine | | Remarks |
				gamma	Date	
	cc.	per cent	mgm.	per cent		
March 1	1300	3.2	.042	16.9	March 1	
March 2	810	6.75	.053			
March 3	1260	2.85	.036	43.0	March 4	Menses began March 3 at
March 4	1300	8.3	.108			9:00 a.m.
March 5	860	13.3	.112			
March 6	500	9.5	.048			
March 7	1590	3.6	.057			Menses ended March 7
March 8	1490	4.3	.065	26.4	March 9	
March 9	1440	2.85	.041			Basal metabolic rate on
March 10	1280	4.3	.055			March 9 plus 10, pulse 84,
March 11	1180	11.3	.133			temperature 98.6, rate 14,
March 12	1260	11.2	.141	9.7	March 13	blood pressure 118/68
March 13	1030	198.0	2.030			Thyroidectomy March 13
March 14	540	225.0	1.210			in a.m.
March 15	875	65.0	.569			
March 16	Lost					
March 17	1280	9.2	.118	8.3	March 18	
March 18	2150					
March 19	1120	4.0	.045			
March 20	970	4.2	.041			
March 21				9.4	March 21	Dismissed

* 24 hours beginning with date indicated.

Laboratory investigation revealed a normal urine, negative Wassermann and Kahn reactions, a normal blood picture and a normal electrocardiogram. The blood iodine on entrance was 16.9 gamma per cent. The blood calcium was 8.2 and the blood phosphorus was 3.4 mgm. per cent. There were no other sig-nificant findings.

She was prepared for thyroidectomy by rest alone. *No iodine was used or administered* throughout the entire management. The daily twenty-four hour excretion of iodine in the urine is presented in Table I. This ranged between 36 and 65 gamma daily, save on two occasions. During early menstruation the daily loss was increased to 108 and 112 gamma. Previous to the thyroidectomy there also occurred an increased output, 133 and 141 gamma. The blood iodine

mostly small, with a small amount of internodular glandular tissue. There was evidence of edema, calcification, fibrosis and hemorrhage. Microscopically a "marked lymphocytic infiltration" was noted. There ensued but a slight reaction, and an otherwise uneventful convalescence.
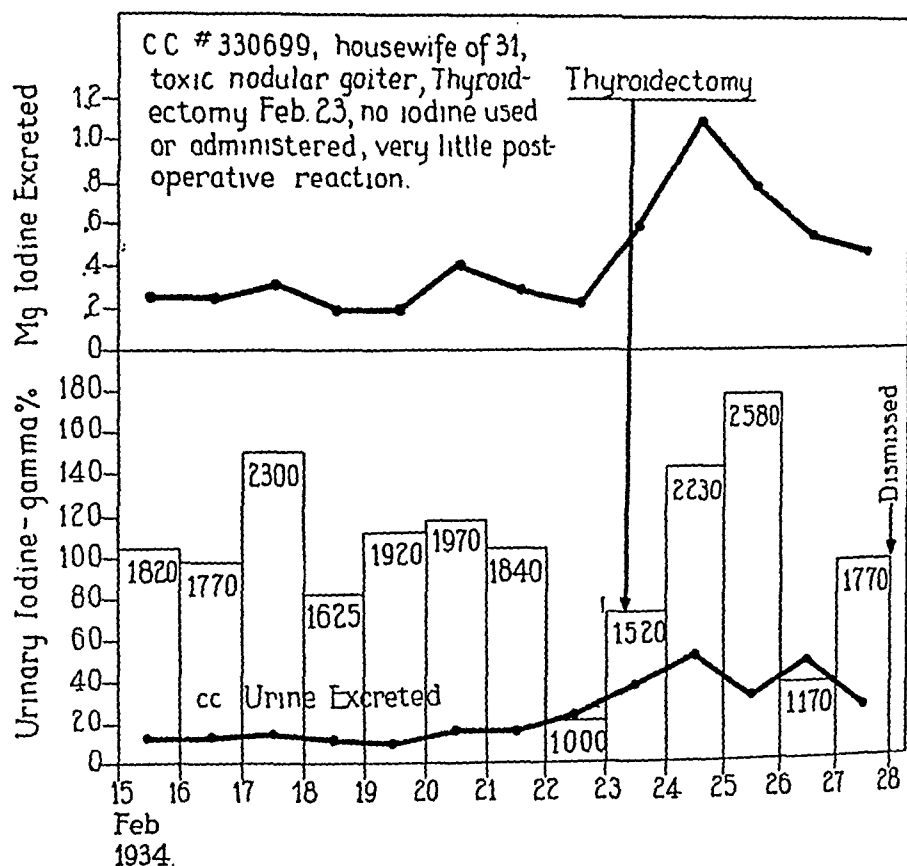
FIG. 3. THE URINARY EXCRETION OF IODINE IN TOXIC NODULAR GOITER

The most marked increase in the postoperative urinary excretion of iodine occurred during the second twenty-four hour period, Figure 3. During the five postoperative twenty-four hour periods the iodine loss ranged from 457 to 1117 gamma (.457 to 1.117 mgm.), averaging 704 gamma (.704 mgm.). On February 28, the blood iodine was 9.2 gamma per cent. The basal metabolic rate subsequently fell to minus 4.

Comment. The preoperative iodine loss was greatly increased. No notable change occurred just preceding the thyroidectomy. The greatest postoperative loss occurred during the second twenty-four hour period. The loss remained high, and did not return to normal in five days. There ensued, following thyroidectomy, an increase in the urinary output and in the iodine concentration.

Patient 4. Diffuse colloid goiter (Table I)

M. S., No. 330981, a housewife of 29, entered the University Hospital, February 28, 1934, for thyroidectomy. She presented a diffuse symmetrical goiter,

hyperthyroidism began about three months previously, with the usual symptoms and a severe weight loss. The thyroid was diffusely and symmetrically enlarged. No nodules were palpable. There were no mechanical symptoms.

She was hospitalized on January 4, 1934, and eight days of bed rest preceded this study. *No iodine was used or administered* throughout her management. On January 6 her basal metabolic rate was plus 40, with the pulse 96, temperature 99° F., respirations 17, and the blood pressure 135/75 during the basal state. The trachea and larynx were normal. The heart revealed only a sinus tachycardia.

Laboratory investigation revealed a normal urine, negative Wassermann and Kahn reactions, essentially a normal blood picture, and an elevated blood iodine. On January 13 the blood iodine was 29.1 gamma per cent. There were no other unusual findings on chemical examination of the blood.

The preoperative urinary excretion of iodine was increased. After a preliminary period of bed rest and adjustment it varied from 55 to 165 gamma daily, during a five day period, averaging 106 gamma per twenty-four hour period. During the twenty-four hours just preceding the operation there was a striking increase, to 949 gamma.

Preparation for thyroidectomy consisted of bed rest alone. Thyroidectomy was accomplished on January 18, using avertin-nitrous oxide anesthesia. Thirty-three grams of vascular, diffuse hyperplastic goiter were resected, leaving a portion estimated as of about 12 grams. The goiter was of the same consistency throughout. No nodules were evident. The cut surface appeared *dry*, rather than moist as are the cut surfaces of similar goiters after preoperative lugolization. Microscopically the sections revealed the "characteristic diffuse hyperplasia." There ensued a moderately severe reaction followed by an otherwise uneventful convalescence.

During the twenty-four hours subsequent to the thyroidectomy there ensued a great increase in the loss of iodine in the urine, Table II. This gradually subsided during the five days succeeding the thyroidectomy, but was elevated, 190 gamma, on the day of dismissal. The average daily urinary excretion of iodine during the five postoperative days was 543 gamma. During this interval 2.7 mgm. of iodine was lost in the urine. The basal metabolic rate subsequently fell to plus 4. The blood iodine on dismissal was 12.5. It subsequently fell to 10.7 gamma per cent.

Comment. The preoperative loss of iodine in the urine was increased. There was a great increase in the loss just preceding thyroidectomy. This may have been due to the excitement of the impending operation. A greater increase ensued following thyroidectomy. There was a decrease in the output of urine. The behavior of the blood iodine, Table II, is characteristic (2).

COMMENT

A summary of the preoperative, as compared to the postoperative daily loss of iodine in the urine, is presented in Table III. The average daily loss of the five patients ranges from 0.062 to 0.269 mgm. The grand average is 0.171 mgm. Following thyroidectomy, the daily loss, at its maximum, is from 1.117 mgm. to 2.030 mgm. The average daily postoperative loss, of the five patients, ranges from 0.521 mgm. to 0.793 mgm.

was elevated to 43.0 gamma per cent during the onset of menstruation. It was 9.7 the morning of the thyroidectomy.

Thyroidectomy was performed on March 13, under avertin-nitrous oxide anesthesia. One hundred grams of diffuse colloid goiter were resected, leaving a portion estimated as of about 18 grams. The goiter was of the same consistency throughout. No nodules were evident. Microscopically it was "struma diffusa colloides, microfollicularis et macrofollicularis." The iodine content of the goiter removed was 56.4 mgm. per cent (dry basis). There ensued a moderate reaction and an otherwise uneventful convalescence.

The usual postoperative increase in the urinary excretion of iodine is presented in Table I. Over two milligrams were lost during the twenty-four hours following the thyroidectomy. There ensued a progressive return to normal and during the two days preceding dismissal there was a normal loss of iodine in the urine. The basal metabolic rate subsequently fell to minus 8. The blood iodine on dismissal, March 21, was 9.4 gamma per cent.

Comment. The preoperative excretion of iodine was within normal range, save in two instances. Once, during menstruation, and again just preceding thyroidectomy, it was increased. A striking iodine loss, followed by a prompt return to normal ensued after thyroidectomy. The behavior of the blood iodine during menstruation is characteristic (13).

Patient 5. Toxic diffuse hyperplastic goiter (Table II). *Exophthalmic goiter*

J. B., No. 326974, a housewife aged 32, was originally seen in the Dispensary, in January, 1934, presenting the picture of moderate exophthalmic goiter. At this time her basal metabolic rate was plus 32. Goiter had long been present, and there was evidence of previous toxicity. The present exacerbation of

TABLE II

Twenty-four hour urine iodine in Patient 5
(J. B., No. 326974, housewife of 32, exophthalmic goiter, no iodine used or administered, moderately severe postoperative reaction, uneventful convalescence)

Date*	Vol-ume	Iodine gamma	Iodine excreted	Blood iodine		Remarks
				Gamma	Date	
	cc.	*per cent*	*mgm.*	*per cent*		
January 12	400	18.8	.075	29.1	January 13	
January 13	650	34.6	.165			
January 14	1380	9.3	.128			
January 15	1250	8.9	.109	20.2	January 16	
January 16	1900	2.9	.055			
January 17	1650	57.5	.949	15.5	January 18	
January 18	1880	79.6	1.497			Subtotal thyroidectomy
January 19	450	113.1	.509	13.2	January 20	January 18 in a.m.
January 20	270	74.0	.200			
January 21	480	63.7	.316			
January 22	710	26.8	.190	12.5	January 23	

* 24 hours beginning with date indicated.

Altogether, we would consider a tissue loss, extrathyroid in nature, to account, at least for some of the excess iodine excreted. This opens up interesting problems concerning the function of iodine in man. It is possible that iodine has a rôle separate from that of furnishing the high iodine content of the thyroid hormone.

CONCLUSION

Subsequent to thyroidectomy there ensues a great increase in the loss of iodine in the urine. Evidence at hand warrants the conclusion that a part of this iodine comes from the extra-thyroid tissues.

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TABLE III

Loss of iodine in the urine following thyroidectomy

Patient number	Diagnosis	Preoperative daily iodine excretion		Postoperative daily iodine excretion	
		Range	Average	Range	Average
		mgm.	*mgm.*	*mgm.*	*mgm.*
1	Non-toxic nodular goiter	.051 to .073	.064	1.366 to .157	.521
2	Mildly toxic nodular goiter	.085 to .276	.201	1.534 to .249	.798
3	Toxic nodular goiter	.196 to .415	.269	1.117 to .457	.704
4	Diffuse colloid goiter	.036 to .141	.074	2.030 to .041	.669
5	Exophthalmic goiter	.055 to .949	.247	1.497 to .190	.543
		Grand average, preoperative	.171	Grand average, postoperative	.647

This grand average is 0.647 mgm. It is thus evident, that following thyroidectomy for goiter, there ensues a great increase in the loss of iodine in the urine.

In the three patients with toxic goiter there is a definite increase in the preoperative loss of iodine. We have noted this finding elsewhere (1) (5) (9). The other two show a normal range of urinary excretion of iodine. In Patient 4 there occurred an increase in the iodine loss during menstruation. Cyclic variations of the elevation of the blood iodine, and in the daily loss of iodine in the urine, may occur in women (13).

DISCUSSION

The source of the excess iodine, lost in the urine subsequent to thyroidectomy, has been of interest. Manipulation of the gland during removal, and absorption from the raw cut-surfaces, were, at first, regarded as likely possibilities. After resection a certain amount of necrosis of the injured areas ensues. The fluid exudate, which collects about the remaining portions of the gland, has a considerable iodine content. Resorption of this may account for some of the iodine loss. On the other hand, an increased urinary loss follows total thyroidectomy (8), in which procedure no raw areas of thyroid are left behind. Manipulation remains an uncontrolled factor in total thyroidectomy.

However, an increased loss of iodine in the urine follows other operations than thyroidectomy, on parts of the body remote from the thyroid (10). Thus, 1.010 mgm. was excreted during the twenty-four hour period following an astragalectomy. 1.750 mgm. was lost during the first twenty-four hour period following an herniorraphy. 2.515, 2.208 and 3.109 mgm. were recovered in the daily urines subsequent to thoracoplasties.

Altogether, we would consider a tissue loss, extrathyroid in nature, to account, at least for some of the excess iodine excreted. This opens up interesting problems concerning the function of iodine in man. It is possible that iodine has a rôle separate from that of furnishing the high iodine content of the thyroid hormone.

CONCLUSION

Subsequent to thyroidectomy there ensues a great increase in the loss of iodine in the urine. Evidence at hand warrants the conclusion that a part of this iodine comes from the extra-thyroid tissues.

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THERAPEUTIC EFFECT OF TOTAL ABLATION OF NORMAL THYROID ON CONGESTIVE HEART FAILURE AND ANGINA PECTORIS.[1] IX. POSTOPERATIVE PARATHYROID FUNCTION. CLINICAL OBSERVATIONS AND SERUM CALCIUM AND PHOSPHORUS STUDIES

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The occurrence of tetany as an infrequent postoperative complication following subtotal removal of the abnormal thyroid gland is well recognized (1) (2). McCullagh in 1932 (2), reported an incidence of tetany of 1.3 per cent in a series of 11,500 cases in which thyroidectomy was performed at the Cleveland Clinic.

The anatomical proximity of the parathyroid glands to the thyroid, the similarity of the signs and symptoms of postoperative tetany to those of idiopathic hypoparathyroidism, and the specific effect of calcium therapy. offer strong evidence that tetany following thyroid surgery is due to removal of, or injury to, the parathyroid glands during operation. Means and Richardson (1) observed that the frequency of postoperative parathyroid tetany depends roughly upon the amount of thyroid tissue removed. Thus, tetany rarely occurs after unilateral thyroidectomy; and is less common after the subtotal removal of adenomatous goitre than after that of exophthalmic goitre (1).

Blumgart and his associates (3) (4) (5) (6) have shown that in patients without thyroid disease, complete removal of the normal thyroid must be performed to assure a persistently subnormal basal metabolic rate. To accomplish complete ablation of the thyroid gland, the region of the parathyroid bodies must be deliberately invaded. It was feared, therefore, that intractable tetany might frequently follow this operation.

The present communication is a report of our clinical observations, and studies of serum calcium and serum phosphorus in relation to parathyroid function following the total removal of the normal thyroid gland for the relief of intractable heart disease and other conditions.[2] Studies of the

Clinical manifestations of tetany following total thyroidectomy

Signs or symptoms of tetany appeared between the first and third day after total thyroidectomy in eight of the patients; in the other four patients signs or symptoms were first manifest on the fifth postoperative day.

The most severe signs and symptoms were encountered in Case 6 (Case reports) and consisted of tingling numbness of the feet and hands and paresthesias of the face and legs, which were accompanied by a feeling of stiffness of the jaw and ankles. This patient also complained of a " smothering " sensation, nausea and extreme apprehension. Chvostek's and Trousseau's signs were active. Within an hour after intravenous injection of 20 cc. of 10 per cent solution of calcium chloride, all of her signs and symptoms disappeared. There was no recurrence except for transient paresthesias of the face and tips of the fingers which occurred on the thirteenth postoperative day, three days after the omission of oral medication with calcium chloride. Case 2 (Case reports) who suffered from none of the symptoms of tetany has been included in this series because a mildly positive Chvostek's sign, which had previously been negative, was elicited on the second postoperative day and persisted for approximately a week. Case 5 (Case reports) experienced only transient paresthesias of the hands and face lasting two days and disappearing without medication; Chvostek's and Trousseau's signs were absent at all times. The severity of tetany in the remaining nine patients varied between that encountered in Case 6 and the mild signs and symptoms observed in Cases 2 and 5 (Case reports).

Trousseau's sign was elicited in six and Chvostek's sign in eleven of the twelve patients with postoperative tetany (Table I). The most active Chvostek's and Trousseau's signs were elicited in those patients who experienced the most severe paresthesias.

The manifestations of tetany were usually temporary, disappearing within the first two postoperative weeks in ten of the twelve patients. The remaining two patients, one of whom was operated upon nine months ago (Case 7), the other two and a half months ago (Case 12) still have some of the symptoms of tetany whenever the amount of calcium administered is reduced.

Treatment of patients with signs and symptoms of postoperative tetany

Three patients with extremely mild clinical symptoms of tetany received no medication and recovered spontaneously two to six days after onset (Cases 5, 8 and 9). Oral administration of calcium chloride solution (35 per cent) in amounts from 4 to 16 cc. four times a day (2 to 8 grams of calcium daily) was usually adequate to control the symptoms of tetany; in three cases an initial dose of 10 to 20 cc. of 10 per cent calcium chloride solution was given intravenously. Occasionally, when symptoms persisted, a fifth oral dose of calcium chloride solution was given during the night. Because of its acidifying properties, calcium chloride has usually been em-

ployed; calcium lactate or gluconate has been substituted when the chloride was not tolerated. Besides the calcium medication, these patients have been given a quart of milk daily as a palatable means of supplying additional calcium. In the first patient of our series to develop tetany (Case 1), 1 cc. of parathormone was administered intramuscularly immediately on recognition of symptoms; viosterol (250 D) 0.5 cc. every six hours was given to the first three cases in the series (Cases 1, 2 and 3) on recognition of signs and symptoms of hypoparathyroidism, and viosterol in varying doses has been administered to Cases 7 and 12, as an adjunct to calcium therapy in these persistent cases.

In the first few patients who developed postoperative tetany, calcium administration was continued for several months after the disappearance of all signs and symptoms. In the later cases, medication was discontinued whenever possible within a few weeks after operation and before the patient left the hospital. In the two patients who, at times, still suffer from symptoms, oral medication with calcium chloride and viosterol are continued.

Studies of serum calcium and phosphorus in patients who developed tetany following total thyroidectomy

The concentration of calcium in the serum at the onset of signs and symptoms of parathyroid insufficiency was variable (Table I). The calcium concentration was less than 7 mgm. per 100 cc. of serum during the period when clinical signs and symptoms were present in only four of the twelve patients, while in six patients the calcium values were above 8.0 mgm. per 100 cc. (Table II). In Case 11 (Table I), for example, the serum calcium was 9.2 mgm. per 100 cc. at a time when the patient was experiencing paresthesias of the hands, feet and face, a sense of pressure over the chest, was apprehensive, and showed moderate Chvostek's and Trousseau's signs. Likewise, in Case 3, the clinical diagnosis of tetany was definite, although the serum calcium was normal. In Case 1, the serum calcium was 9.2 mgm. per 100 cc. (Clarke's method) one hour after the administration of parathormone, at which time the patient still experienced symptoms of tetany.

The concentration of inorganic phosphorus in the serum during early postoperative tetany was 5.0 mgm. per cent or more in only two cases (Cases 1 and 7), the highest value for phosphorus obtained being 5.6 mgm. per 100 cc. (Case 7; Table II). In Case 8 the serum phosphorus increased from a preoperative value of 3.7 mgm. per 100 cc. to 4.9 mgm. per 100 cc. on the eighth postoperative day. In all other instances the concentrations of serum phosphorus were normal, and not significantly different from the preoperative values throughout the tetany period.

Measurements made at intervals during the first year after operation, in those patients who showed tetany lasting only two weeks or less, re-

vealed a persistent slight decrease in serum calcium as compared with the preoperative level. Thus, in six cases in which measurements were made before and six months after operation, the average serum calcium value at the sixth postoperative month was 8.4 mgm. per 100 cc. as compared with the average preoperative value of 9.3 mgm. per 100 cc. (Table II).

The serum protein concentration was normal in two cases in which the serum calcium was very low on the second to fifth postoperative days. These, and other measurements of serum protein made during the early postoperative period (Table II) demonstrate that the decrease of serum calcium observed during this period was not dependent upon decreased protein concentration. Similarly, the slightly diminished concentration of serum calcium found in certain cases six months after operation was not dependent upon a decreased serum protein.

Studies of serum calcium and phosphorus in patients who did not develop tetany following total thyroidectomy

An appreciably decreased calcium concentration in the serum was evident in nine of thirteen cases, none of which developed clinical signs or symptoms of tetany following total thyroidectomy (Table II). The greatest decrease in serum calcium usually occurred during the second to fifth days after operation; the maximum decrease observed was 2.7 mgm. per 100 cc. (Case 24, Table II), the average decrease 1.2 mgm. per 100 cc. In several instances the calcium concentration decreased to values between 7.1 and 7.7 mgm. per 100 cc. The values for serum phosphorus following total thyroidectomy did not differ from those obtained before operation (Table II). No appreciable decreases in serum protein were observed during this early postoperative period (Table II).

Measurements in this group during the interval from one month to one year following total thyroidectomy revealed a persistent but slight decrease in serum calcium concentration in most instances (Table II). Thus, the average value for calcium six months after operation in the nine cases studied was 8.8 mgm. per 100 cc. compared to the preoperative average of 9.6 mgm. per 100 cc. The extent of these changes was similar to that observed at the same postoperative period in the cases which showed transient clinical signs of tetany early in the postoperative course.

The concentration of inorganic phosphorus in the serum was not significantly different from the preoperative value at any time after operation. The concentrations of serum protein six months after operation showed definite increases over the preoperative values in many instances and no appreciable changes in the other cases (Table II). The slightly lowered concentrations of serum calcium observed six months after operation were, therefore, not dependent upon decreased concentration of serum protein.

development of more serious complications; the actual duration of the parathyroid deficiency is probably not affected.

Albright and his associates (13), and Ellsworth (14) have pointed out that low serum calcium and high serum phosphorus values are cardinal features of parathyroid tetany. The patients studied by Albright (13) and Ellsworth (14) were cases of long standing hypoparathyroidism of idiopathic or postoperative etiology. Case 7 of our series showed changes in serum calcium and phosphorus one month after operation similar to those observed in chronic tetany (15) (16), the serum calcium concentration being 5.9 mgm. and the serum phosphorus 5.6 mgm. per 100 cc. However, our results show that at the time of onset of clinical signs and symptoms of parathyroid tetany in man, blood chemical findings characteristic of chronic hypoparathyroidism may not be present. In contrast to our findings in man, studies of others on parathyroidectomized dogs show that the serum calcium is, almost without exception, strikingly decreased and the serum phosphorus increased at the time of onset of symptoms of tetany (15) (16) (17).

The finding of a normal concentration of inorganic phosphorus in the serum at the time of onset of postoperative tetany in most of our cases was unexpected, in the light of the above considerations. There are, however, a few observations early in the course of postoperative tetany in man which accord with this finding (18) (19). Bauer, Marble and Claflin (18) observed carpopedal spasm three days after a second stage removal of the thyroid in a patient in whom the concentration of serum inorganic phosphorus was only 4.6 mgm. and the concentration of serum calcium 6.8 mgm. per 100 cc. Two weeks after operation the serum inorganic phosphorus in this patient was 6.7 mgm. and the serum calcium 5.2 mgm. per 100 cc. Bauer and his associates (18) pointed out that the concentration of calcium was abnormally low in this patient at the time when carpopedal spasms were first manifest, but that only subsequently did the concentration of serum inorganic phosphorus rise to an abnormally high level. Likewise, Taubenhaus (19) observed a patient who developed symptoms of severe tetany five days after subtotal thyroidectomy, at which time the inorganic phosphorus of the serum was 5.0 mgm. and the calcium 5.4 mgm. per 100 cc. Our findings and those of Bauer (18) and Taubenhaus (19) make it clear that the change in serum calcium in acute hypoparathyroidism may precede the change in serum phosphorus. This is in contrast with the primary change in serum phosphorus in acute hyperparathyroidism (13) (14) (23).

Our observations show that while studies of serum calcium and phosphorus are of great value in the diagnosis and management of postoperative tetany, the clinical signs and symptoms are of the utmost importance. McCullagh (2) observed that the clinical diagnosis of postoperative tetany is sometimes not corroborated by the serum calcium findings. He sug-

gested that the clinical diagnosis of tetany in such cases may have been incorrect, and noted that some normal individuals may have a mildly positive Chvostek's sign.   In view of our observations, however, we believe that the early diagnosis of postoperative tetany can be made definitely without the confirmatory evidence of abnormal chemical findings.   Although Chvostek's sign was mild in several of our patients, it appeared to be significant because of its clearly defined onset and disappearance.   Experience in other clinics has shown that if postoperative parathyroid tetany is not diagnosed and treated early, convulsions may ensue, and death may follow (1).

It has been established, beyond reasonable doubt, that a sudden marked decrease in the physiologically active fraction of the serum calcium leads to the symptom complex of tetany in man, whereas some patients with chronic hypoparathyroidism may have no symptoms for years, in spite of markedly diminished concentrations of calcium in the serum (11) (20) (21).   The cause of tetany is not clear in those of our patients who showed clinical signs and symptoms without a significant reduction in the total concentration of calcium in the serum.   It may be that the amount of the sudden diminution of the active fraction of the serum calcium which is sufficient to precipitate mild tetany is, in certain cases, too small to be detected by the usual measurement of total serum calcium.   With the persistence of the physiological derangement a sustained significant decrease in the active fraction of the serum calcium may, however, become apparent through a decrease in the concentration of total calcium.   Studies of the concentration of magnesium in the serum during early postoperative tetany following total thyroidectomy are being made by Miss Dorothy Tibbetts at the Huntington Memorial Hospital, Boston.   Kruse, Orent and Mc-Collum (22) have recently demonstrated that tetany occurs in dogs with low concentrations of serum magnesium, but that the syndrome of this deficiency is different from that observed in parathyroidectomized animals (22).

Rabinowitch (24) found decreases in the concentration of serum calcium of 1.3 mgm. to 3.5 mgm. per 100 cc. during the first week following subtotal thyroidectomy in ten of thirteen thyrotoxic patients, none of whom developed tetany after operation.   In most of these cases the serum calcium returned to the preoperative level by the end of the second week after operation (24).   These observations of Rabinowitch following subtotal thyroidectomy (24) are similar to ours of a decreased concentration of serum calcium during the period immediately following total thyroidectomy in nine of thirteen cases, none of whom developed clinical signs or symptoms of reduced parathyroid function.   The concentration of inorganic phosphorus in the serum remained normal in these patients throughout the postoperative period; the decrease of serum calcium was not attributable to a lowering of the concentration of serum protein (Ta-

ble II). From our findings after total thyroidectomy, and those of Rabin-owitch after subtotal thyroidectomy, it appears that mild transient parathyroid insufficiency occurs in approximately 80 per cent of patients following these procedures. In patients who show such changes in serum calcium without symptoms of tetany the decreased calcium is of no clinical importance.

The persistence of slightly subnormal values for serum calcium for months following total thyroidectomy, both in patients who showed transient clinical signs of hypoparathyroidism and in those who showed no signs, is probably not dependent upon the hypothyroid state induced (as evidenced by low basal metabolic rates (Table II)), since the serum calcium in cases of myxedema of idiopathic origin has been found to be normal (25). Our results suggest that some thyroidectomized patients may have a slight reduction in parathyroid function for several months after operation although no clinical manifestations are evidenced.

It is probable that one of our patients, in whom symptoms of tetany have been manifest at various times during the nine months since operation (Case 7), will continue to show clinical evidence of parathyroid deficiency whenever medication is discontinued. Indeed, even when this patient is receiving large amounts of calcium salts, she occasionally experiences mild paresthesias. The efficacy of large doses of viosterol as an adjunct to calcium therapy (18) in this case is being studied; parathyroid extract has not been employed, since its effectiveness has been shown to be only temporary (26). In the other patient of this series who still shows clinical signs of reduced parathyroid function two and a half months after operation (Case 12), the symptoms are satisfactorily controlled by oral administration of calcium chloride, supplemented by viosterol.

SUMMARY

1. Tetanic convulsions or spontaneous spasm of the extremities did not occur in any of seventy-three consecutive patients on whom total thyroidectomy was performed. Clinical signs or symptoms of mild parathyroid deficiency were manifest after operation in twelve patients, or 17 per cent, of this entire series. Of the last thirty-seven patients of this group of seventy-three, only three, or 8 per cent, showed signs or symptoms.

2. In ten of the twelve patients clinical signs and symptoms of hypoparathyroidism were transient, disappearing within two weeks. One patient who was operated upon two and a half months ago, and another nine months ago, still show signs and symptoms when specific medication is discontinued.

3. The symptoms of hypoparathyroidism are attributed to injury, rather than to removal of parathyroid glands during operation.

4. Oral administration of calcium chloride solution and a diet rich in milk controlled the symptoms of tetany in most patients in whom the

disease was transient.   An initial intravenous injection of calcium chloride solution was given to three patients; calcium lactate or gluconate was substituted when oral administration of calcium chloride solution was not tolerated.   Viosterol, together with a large intake of calcium, is being employed successfully in the two cases with persistent hypoparathyroidism.

5. The serum calcium was reduced to 7.5 mgm. per 100 cc. or less in six of the twelve cases at the time of onset of tetany; in three cases the serum calcium was between 8.3 and 8.6 mgm. per 100 cc.; in the remaining three cases the serum calcium was within the accepted normal limits. The values for serum inorganic phosphorus in these patients with early postoperative tetany were usually normal, being 5.0 mgm. per cent or above in only two cases.

6. Appreciable decreases in concentration of serum calcium and no changes in concentration of serum inorganic phosphorus were observed during the first two weeks after total thyroidectomy in a group of patients who showed no clinical signs of insufficient parathyroid function.

7. The concentration of serum calcium was usually slightly below the preoperative level during the first year after thyroidectomy, both in those individuals who showed transient signs and symptoms of hypoparathyroidism soon after operation and in patients who showed no clinical signs of this disorder at any time.

8. It is pointed out that the chemical changes in the blood present during the early stages of postoperative tetany may be quite different from the characteristic markedly low serum calcium and high phosphorus values found in chronic hypoparathyroidism of either idiopathic or postoperative origin.

9. The transient tetany which sometimes occurs immediately following total thyroidectomy can be controlled by calcium therapy; persistent parathyroid insufficiency occurs so rarely that it does not constitute a contraindication to total thyroidectomy.

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CASE REPORTS

Case 1. Rheumatic heart disease; mitral stenosis; congestive failure; mild, transient tetany following total thyroidectomy. B. Z., a housewife, aged 42, was operated upon April 8, 1933. The early postoperative course was uneventful. On the third postoperative day the patient complained of tingling numbness in the face and hands, most marked in the tips of the fingers, and showed moderate Chvostek's and Trousseau's signs. The patient stated that numbness of the face and hands had gradually increased during the preceding two days, but that the symptom was so vague that she did not complain even on questioning. Chvostek's and Trousseau's signs were not elicited during these two days. Immediately on recognition of symptoms of tetany, the nurse in charge administered 1 cc. of parathyroid extract (Collip) intramuscularly in accordance with standing orders. The serum calcium one hour after injection was 9.2 mgm. (Clarke's method) and the serum phosphorus 5.0 mgm. per 100 cc. Following the injection of parathyroid extract, 4 cc. of 35 per cent calcium chloride solution each hour and 0.5 cc. of viosterol, 250 D, were administered orally. Within a few hours after medication was instituted, the clinical signs and symptoms disappeared. For the next two days the patient received 4 cc. of 35 per cent calcium chloride solution every two hours during waking hours, every three hours during the night, and 0.5 cc. of viosterol every six hours, with no recurrence of signs or symptoms. Blood drawn on these two mornings, three hours after calcium chloride medication, showed calcium values of 14.0 and 13.8 mgm. per 100 cc. of serum respectively (Clarke's method). On the following morning, after 72 hours of therapy, the serum calcium had increased to 16.6 mgm. per 100 cc. (Clarke's method) and the serum phosphorus decreased from a preoperative level of 4.1 to 2.7 mgm. per 100 cc. Medication was therefore omitted. On the tenth postoperative day, four days after the cessation of therapy, the patient again complained of numbness and tingling of the hands and face. Moderately active Chvostek's and Trousseau's signs were elicited. The serum calcium was 9.4 and the serum phosphorus 3.6 mgm. per 100 cc. Calcium chloride, 4 cc. of 35 per cent solution every two hours, and viosterol 0.5 cc. every six hours, were again administered. All signs and symptoms of tetany disappeared late that day.

During the next few weeks the viosterol was gradually omitted and the calcium medication was reduced to 8 cc. of 35 per cent solution calcium chloride twice daily together with a quart of milk daily. The fasting serum calcium concentration remained around 8.2 mgm. per 100 cc. The lack of clinical significance of this slightly subnormal serum calcium was not appreciated at this time and therefore medication was continued until the sixth postoperative month. To the time of writing, approximately one year after operation and six months after cessation of calcium therapy, the patient has experienced no recurrence of signs and symptoms of tetany.

Case 2. Rheumatic heart disease; mitral stenosis; congestive failure; mild, transient tetany following total thyroidectomy. W. D., a man, aged 23, was operated upon April 15, 1933. The early postoperative course was uneventful.

On the second postoperative day a mild Chvostek's sign was elicited; no symptoms of tetany were manifest, however. Four cc. of 35 per cent calcium chloride solution every three hours was administered and on the fourth day after operation, because of the persistence of a moderate Chvostek's sign, 0.5 cc. of viosterol every six hours was added to the calcium therapy. The serum calcium on the third postoperative day was 8.6 mgm. per 100 cc. (Clarke's method). On the fifth postoperative day, the dose of calcium chloride was decreased to 4 cc. four times a day and once during the night. Viosterol was decreased to 0.5 cc. twice daily. No further signs or symptoms of tetany were manifest except on one occasion, the eleventh postoperative day, when a mild Chvostek's sign was again elicited. One month after operation, viosterol was discontinued and the calcium chloride medication gradually decreased until finally omitted approximately two months after operation. The serum calcium nine months after operation was 8.3 mgm. per 100 cc. It is now one year since operation and no signs or symptoms of tetany have been manifest since the disappearance of the Chvostek sign on the eleventh postoperative day.

Case 3. Rheumatic heart disease; mitral stenosis; congestive failure; mild, transient tetany following total thyroidectomy. L. B., a housewife, aged 45, had a total thyroidectomy April 17, 1933. The early postoperative course was uneventful. On the fifth day after operation the patient experienced tingling numbness of the fingers and in the vicinity of the operative wound. A mild Chvostek's sign was elicited but Trousseau's sign was negative. The serum calcium at this time was 10.7 mgm. (Clarke's method). She received eight cc. of 35 per cent calcium chloride solution orally every two hours during waking hours and every four hours at night and 0.5 cc. of viosterol every six hours. On the following day the signs and symptoms of tetany had disappeared and medication was discontinued. There was no recurrence of symptoms until about ten days later when the patient complained of a vague, numb sensation in the fingers; Chvostek's and Trousseau's signs were negative at this time. Although we did not believe this symptom was due to parathyroid tetany, one dose of 8 cc. of 35 per cent calcium chloride solution was given orally, and subjective numbness disappeared. Up to the time of the present writing, approximately one year after operation, the patient has had no recurrence of signs or symptoms of tetany.

Case 4. Arteriosclerotic heart disease; coronary sclerosis; angina pectoris; mild, transient tetany following total thyroidectomy. E. P., a woman, aged 58, was operated upon July 1, 1933. The early postoperative course was uneventful. No signs or symptoms of tetany were evident until the fifth postoperative day, when she experienced tingling numbness of the fingers, involuntary twitching of the mouth, and stiffness of the body. At this time a moderate Chvostek's and a moderate Trousseau's sign were elicited. The serum calcium was reduced to 7.2 mgm. per 100 cc.

Eight cc. of 35 per cent calcium chloride solution were administered every three hours, and a quart of milk was taken daily. She was unable to retain the calcium chloride solution, and the signs and symptoms of tetany, though somewhat diminished, persisted during this and the following day. On the next day she received four ounces of 4 per cent calcium gluconate solution every three hours, the quart of milk daily being continued. That day the signs and symptoms of tetany disappeared, and did not recur until five days later, when the patient again complained of transient numbness of the fingers. At this time Chvostek's and Trousseau's signs were negative; the fasting serum calcium was still reduced to 7.2 mgm. per 100 cc. During the next two weeks the serum calcium rose to 8.0 mgm. per 100 cc. and medication was omitted. At the time

of writing, nine months after thyroidectomy, the patient still remains free of clinical signs and symptoms of parathyroid insufficiency.

Case 5. Generalized arteriosclerosis; arteriosclerotic heart disease; congestive failure; mild, transient tetany following total thyroidectomy. J. T., a man, aged 59, was operated upon July 25, 1933. The early postoperative course was uneventful. On the fifth day after operation he complained of occasional tingling of the right hand and of the nose. On the following day he noted mild transient stiffness of the jaw and numbness of the face. These symptoms disappeared on the same day without medication. Chvostek's and Trousseau's signs were negative at all times. The serum calcium two days after operation had decreased to 7.5 mgm. per 100 cc.; calcium measurements were not made on the fifth or sixth postoperative days when the symptoms were present. One month postoperatively the serum calcium had returned to the preoperative level of 8.7 mgm. per 100 cc. Since the sixth postoperative day, no symptoms of tetany have been experienced up to the time of writing, approximately eight months after operation.

· *Case 6. Hypertension; hypertensive heart disease; congestive failure: transient tetany following total thyroidectomy.* B. R., a housewife, aged 48, was operated upon July 28, 1933. The temperature was elevated to approximately 101° F. during the first five postoperative days. During the evening of the third postoperative day the patient noted a numb sensation of the feet. The serum calcium on the morning of this day was 7.8 mgm. per 100 cc. On awakening the following morning she noted tingling numbness of both feet, this sensation gradually increasing to the level of the knees and later to the abdomen. The patient called the nurse and complained of these symptoms for the first time. Numbness and tingling of the face, hands and arms, sensations of " smothering," heaviness over the chest, stiffness of the ankles and jaws, apprehension and nausea rapidly developed. Chvostek's and Trousseau's signs were active. Twenty cc. of 10 per cent calcium chloride solution were administered intravenously. One hour after this injection the symptoms of tetany and the Chvostek's and Trousseau's signs had entirely disappeared. The fasting serum calcium just prior to the calcium chloride injection was 8.3 mgm. per 100 cc. During the remainder of this day and during the following two days 8 cc. of 35 per cent calcium chloride solution were given orally every three hours, 0.5 cc. of viosterol every twelve hours, and a quart of milk daily. The patient experienced no paresthesias, and the medication was reduced on the fourth day after the onset of symptoms to 8 cc. of calcium chloride solution every six hours, and a quart of milk daily. On the tenth day after the onset of symptoms the serum calcium had increased to 9.6 mgm. per 100 cc. and calcium medication was accordingly omitted. On the thirteenth postoperative day the patient noted transient tingling numbness of the tips of the fingers and of the face. Chvostek's and Trousseau's signs were, however, negative. Since this time there has been no recurrence of the signs or symptoms of tetany up to the time of writing, eight months after operation.

Case 7. Rheumatic heart disease; mitral stenosis; congestive failure; chronic tetany following total thyroidectomy. S. B., a housewife, aged 52, was operated upon August 9, 1933. Early on the morning of the second postoperative day a Chvostek's sign was elicited. Two hours later she noted tingling numbness of the right leg and foot and of the skin about the mouth. Active Chvostek's and Trousseau's signs were elicited at this time. The serum calcium was 7.2 mgm. per 100 cc. and the serum phosphorus 5.3 mgm. Ten cc. of 10 per cent calcium chloride solution was administered intravenously, with the disappearance of all signs and symptoms within the next two hours. For the re-

mainder of this day she was given 8 cc. of 35 per cent calcium chloride solution orally every three hours, and for the following three days 8 cc. of 35 per cent calcium chloride solution every eight hours, and a quart of milk daily. Signs and symptoms of tetany did not recur, and medication was omitted on the sixth postoperative day. On the following day the patient experienced numbness of the face; a mild Chvostek's sign was elicited, Trousseau's sign was negative, the serum calcium was 6.3 mgm. per 100 cc. Eight cc. of 35 per cent solution of calcium chloride was again administered every eight hours. On discharge from the hospital, approximately two weeks after operation, she was advised to continue to take this calcium medication and a quart of milk daily.

She was seen three weeks after her discharge, at which time she complained that she had experienced transient numbness and tingling of the extremities and a shaking feeling and stiffness of the limbs on several occasions since her discharge from the hospital, although she stated that she had adhered to the calcium medication as ordered. The fasting serum calcium at this time was 5.9 mgm. per 100 cc., the serum phosphorus 5.6 mgm. per 100 cc., and Chvostek's and Trousseau's signs were active. Calcium medication was increased by the addition of 150 grains of calcium lactate daily.

During the next month the patient intermittently failed to take the calcium chloride solution for a period of a day or two, at which time paresthesias reappeared. From the second to the ninth months after operation, the patient continued to experience mild paresthesias at times, in spite of a high calcium intake. With large doses of viosterol (5 to 15 cc. daily of 250 D) as an adjunct to calcium therapy, she has been relieved of symptoms and the serum calcium has been increased to 8.6 mgm. per 100 cc.

Case 8. Rheumatic heart disease; mitral stenosis; congestive failure; mild tetany following total thyroidectomy. F. D., a man, aged 18, was operated upon August 25, 1933. The temperature rose to 103° F. on the first postoperative day, returning to normal the following day. On the second postoperative day the patient complained of tingling of the fingers. A moderate Chvostek's sign was elicited at this time; Trousseau's sign was negative. On the third postoperative day the patient experienced transient tingling of the right hand; a mild Chvostek's sign was elicited; the serum calcium was 8.3 and serum phosphorus 3.3 mgm. per 100 cc. Transient tingling of the right hand was experienced until the sixth postoperative day, and Chvostek's sign was occasionally elicited until the eighth postoperative day, at which time the serum calcium was 7.7 mgm. per 100 cc. and the serum phosphorus 4.9 mgm. per 100 cc. Up to the present writing, approximately seven months after operation, there has been no recurrence of the signs or symptoms of parathyroid insufficiency.

Case 9. Rheumatic heart disease; mitral stenosis; congestive failure; mild, transient tetany following total thyroidectomy. L. M., a man, aged 28, was operated upon August 23, 1933. The temperature rose to 102° F. on the first postoperative day and gradually subsided to normal by the eighth postoperative day. On the fifth day after operation the patient experienced tingling numbness of the hands and fingers; a moderate Chvostek's sign was elicited, Trousseau's sign was negative, and the fasting serum calcium was reduced to 6.2 mgm. per 100 cc. and the serum phosphorus level was 3.5 mgm. per 100 cc. During the following six days the signs and symptoms gradually disappeared without medication. The serum calcium on the tenth postoperative day, the day before disappearance of signs and symptoms, was still 6.2 mgm. per 100 cc. and the serum phosphorus was 3.8 mgm. This patient showed no recurrence of signs and symptoms of tetany up to the time of his death, due to bronchopneumonia and congestive heart failure, six weeks after operation.

Case 10. Arteriosclerotic heart disease; coronary sclerosis; angina pectoris; mild transient tetany following total thyroidectomy. M. H., a man, aged 54, had a right hemithyroidectomy October 14, 1933, and on January 2, 1934, the remaining thyroid tissue was removed. The temperature rose to 102° F. on the first day after the total ablation of the thyroid and returned to normal on the sixth postoperative day. On the second postoperative day the patient experienced numbness of the fingers; Chvostek's and Trousseau's signs could not be elicited at this time. On the third postoperative day the patient complained of numbness and tingling of the hands, feet and legs. On the fourth day after operation the serum calcium was 6.7 mgm. per 100 cc. The signs increased until the seventh postoperative day, at which time a moderate Chvostek's sign was elicited but Trousseau's sign was still negative. On the eighth postoperative day the serum calcium was still reduced to 6.5 mgm. per 100 cc. and the signs and symptoms did not decrease. Calcium medication was instituted. The signs diminished, but were still evident on the eleventh postoperative day, when the dose of calcium chloride was increased to 12 cc. every three hours, with disappearance of signs and symptoms on the following day. On the thirteenth postoperative day the patient was nauseated by the calcium chloride solution, and medication was omitted. The serum calcium five days after cessation of therapy was 8.4 mgm. per 100 cc. There has been no recurrence of signs or symptoms of tetany to the time of writing, three and a half months after operation.

Case 11. Congenital heart disease; patent ductus arteriosus; congestive failure; mild, transient tetany following total thyroidectomy. G. O., a woman social worker, aged 31, was operated upon January 17, 1934. Late on the first postoperative day the patient experienced numbness of the fingers, hands and feet; Chvostek's and Trousseau's signs were negative. On the second postoperative day she complained of tingling numbness of the hands, feet and face, a sensation of pressure over the chest, coldness of the feet, hot waves over the face, and apprehension. Moderate Chvostek's and Trousseau's signs were elicited. Ten cc. of 10 per cent calcium chloride solution were given intravenously. The serum calcium, before the calcium administration, was 9.2 mgm. and the serum phosphorus 2.7 mgm. per 100 cc. During the first hour after the calcium injection, the symptoms of tetany disappeared, Trousseau's sign became negative, but a mild Chvostek's sign could still be elicited. Eight cc. of 35 per cent calcium chloride solution were given orally four times daily. On the next day all signs and symptoms of tetany had disappeared except for occasional numb sensations of the face. Medication was gradually reduced and finally discontinued in the course of the next six weeks. Following the acute episode on the second postoperative day the patient, up to the time of writing, three months after operation, has had no recurrence of signs and symptoms of tetany, with the exception of occasional transient numbness of the face, which we believe may be due to the other causes. The serum calcium has remained above 9 mgm. per 100 cc. and the serum inorganic phosphorus has been consistently normal.

Case 12. Rheumatic heart disease; mitral stenosis; congestive failure; mild tetany following total thyroidectomy. A. S., a housewife, aged 34, had a total thyroidectomy February 9, 1934. The early postoperative course was uneventful. On the afternoon of the second postoperative day, the patient noted tingling of the feet and hands and numbness of the lips; Chvostek's sign was moderate and Trousseau's sign was mild. Eight cc. of 35 per cent calcium chloride solution orally, and a glass of milk were given every three hours for the rest of this day and evening. Signs and symptoms of tetany persisted until the next morning, at which time the serum calcium was 6.6 mgm. per 100 cc. and the serum phosphorus 3.9 mgm. per 100 cc. The calcium chloride medication was in-

creased to 16 cc. five times a day for the following three days. Attempts to reduce the calcium chloride medication to less than 8 cc. of 35 per cent solution four times a day during the next three weeks resulted in recurrence of mild signs and symptoms. The fasting serum calciums (taken twelve hours after medication) persisted at 6.6 to 6.9 mgm. per 100 cc. during this period. The highest serum phosphorus obtained was only 4.4 mgm. per 100 cc. On discharge from the hospital, approximately one month after operation, this patient was advised to take a quart of milk daily and 8 cc. of 35 per cent calcium chloride solution four times a day. During the following two weeks she experienced mild symptoms on two occasions when she tried to reduce the calcium medication. At the present writing, two and a half months after operation, the patient is free from symptoms on a regimen of 8 cc. of 35 per cent calcium chloride solution three times a day, a quart of milk each day, and 0.5 cc. of viosterol three times a day. The fasting blood calcium concentration at this time is 8.3 mgm. per 100 cc.

OBSERVATIONS ON THE TREATMENT OF PELLAGRA

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During the past few years great interest has centered in the etiology and treatment of pellagra. The disease was first recognized in 1735 by Casál (1), a Spanish physician, who believed that diet was a contributing factor in its development. Since that time, many theories (2, 3, 4) have been advanced to explain its cause but at present only two of them are widely recognized: pellagra may be due to a bacterial infection (4), yet no specific bacterium has been discovered which is accepted by all as the causative agent; the disease, on the other hand, may be strictly a deficiency state (3) but no specific chemical substance has been isolated which prevents its development. It is well known, however, that many cases have been relieved by an adequate diet. On the other hand, many individuals in the advanced stages of the disease do not respond to such a diet while others are unable to take it at all. Likewise, many drugs, minerals, and food substances have been recommended as specific therapeutic agents (3, 5, 6, 7).

With the various theories of etiology and the diversity of recommended methods of treatment in mind, it seemed worthwhile to study pellagra in the hope that some additional information might be added to the present knowledge. This paper presents the results following the use of autoclaved yeast, ventriculin, and parenteral liver extract in a special clinic formed for the study of pellagra.

SPECIAL STUDIES

I. A study of yeast as a therapeutic agent

Goldberger and his associates (3, 12) showed that yeast, when added to a deficient diet, was efficacious in the prevention, and sometimes in the treatment of black tongue in dogs and pellagra in human beings. Later they concluded (3) from studies in prevention that both pellagra and blacktongue were due solely to the lack of vitamin G (anti-dermatitis vitamin) which is the heat stable portion of the vitamin B complex. These investigators (14) also found that many of the common foods prevent the development of blacktongue and pellagra which they consider analogous.

Since autoclaved yeast and many complex foods have been used as supplements to a deficient diet and apparently have protected against the development of pellagra, it was decided to place pellagrins on a diet of autoclaved yeast alone to determine whether it in itself might be used as a food capable of producing a remission of the disease.

Five classical cases of pellagra with involvement of the tongue and oral mucous membranes were chosen for this study. Each patient was given autoclaved yeast [1] daily in amounts ranging from 250 to 500 grams, was allowed water ad libitum, and remained in bed throughout the course of the experiment.

All individuals in this group tolerated the diet of autoclaved yeast. At the end of the second day of the yeast therapy the mouth lesions were distinctly less red and swollen and by the fifth day they had practically disappeared. The patients by this time had ravenous appetites so they were given a high caloric, high vitamin diet. While these observations on the improvement of the oral lesions of five pellagrins restricted to a diet of yeast are in support of previous experiments by Goldberger and his associates (3) who found that supplements of yeast possessed preventive and therapeutic properties, in addition they show that yeast can be used as the sole source of food for pellagrins with rapid remission of the disease. Murlin and Mattill (13) have shown that yeast is an adequate food for human beings and contains such complex materials as proteins, fats, carbohydrates, minerals, and accessory food substances. Studies in this clinic (11) have shown that patients gain weight when they receive a diet consisting only of autoclaved yeast and water.

In view of the numerous recognized constituents of yeast and the variability of the disease, pellagra, any observation at this time must be interpreted with care before a conclusive statement can be justifiably made as to the nature of the substance in the yeast capable of producing therapeutic results. Some observers (12), however, consider the action of such a complex chemical substance as autoclaved yeast the same as that of a specific vitamin and designate this vitamin, G or B_2. Goldberger and his associates (3, 12) have found that large amounts of many foods such as meat, milk, and eggs prevent the development of pellagra when added to a deficient diet and the present results indicate that autoclaved yeast in sufficient amounts can be used as the sole food and will cure the oral lesions of pellagra. Since all the known foods which result in the improvement of pellagrous lesions, including autoclaved yeast, are composed of many different materials, the chemical and physiological actions of which are little understood, it seems unwise at present to say unconditionally that the antipellagric factor is the heat stable substance or substances (vitamin G) which investigators have found so important in animal nutrition. Al-

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[1] Furnished through the courtesy of Dr. Isaac F. Harris, Harris Laboratories, Tuckahoe, N. Y.

though Goldberger and his associates call this substance which protects against pellagra in each foodstuff, vitamin G, there is no proof that it is the same material in each food which is utilized by the body to prevent the disease.

Furthermore, it may well be that various materials are required to prevent such diverse manifestations of pellagra as gastro-intestinal lesions, involvement of the nervous system, and dermatitis. In a previous report (8) it was shown that striking improvement occurred in the skin lesions of five pellagrins, who were restricted to an unbalanced diet containing little of the so-called " vitamin G." This observation has been confirmed by Smith and Ruffin (9). As an explanation for the improvement, Wheeler (16) has suggested that a restricted diet forces a breakdown of the patient's body proteins which are subsequently used to cure the lesions but at the present time the information concerning the pathogenesis of pellagra is inadequate for a satisfactory evaluation of Wheeler's hypothesis.

II. Ventriculin as a therapeutic agent

For some years pellagra and pernicious anemia have been considered special types of deficiency diseases by some investigators. Each disease is characterized by remissions and relapses, glossitis, pyrexia, achylia gastrica, and at times central nervous system involvement, but despite these common signs and symptoms, the co-existence of the diseases has been observed rarely. Soon after Castle demonstrated the fundamental difference between the gastric juice of normal human beings and that of patients with Addisonian pernicious anemia (27), Sharp (17) and Sturgis and Isaacs (18) produced characteristic remissions of pernicious anemia by giving ventriculin, a defatted, dessicated substance prepared from pig's stomach, as Minot and Murphy (19) had been able to do with the oral administration of liver. In view of the fact that pellagra and pernicious anemia are two distinct clinical conditions with many similar signs and symptoms, it seemed worthwhile to determine whether ventriculin would cause remission in pellagra as it does in pernicious anemia.

Eight patients with the classical lesions of pellagra were selected for this determination. Each patient when admitted to the hospital was offered—but not forced to eat—the unbalanced diet low in so-called " vitamin G," shown in Table I. This diet resembles one of Goldberger and Wheeler's more than any of the other deficient diets.

After a period of from two to ten days the glossitis and stomatitis became more severe. At this time ventriculin [2] in amounts of 750 to 1000 grams per day was added to the basal diet of each of five patients and no other food was allowed. Three other patients were given the ventriculin but the basic diet was discontinued.

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[2] Furnished through the courtesy of Dr. E. A. Sharp, Parke, Davis & Co.

TABLE I
Basal diet

Food	Weight grams	Protein grams	Fat grams	Carbo-hydrate grams	Ca grams	Mg grams	K grams	Na grams	P grams	Cl grams	S grams	Fe grams
Breakfast												
Hominy grits*	35	3.3	.1	27.5	.004	.020	.061	.007	.051	.016	.048	.0003
Cornmeal muffins (3 muffins)	15	9.9	11.1	91.2	.019	.048	.161	.051	.138	.108	.148	.0009
Sugar				15.0								
Coffee					15.9	.004	.017	.043	.008	.038	.029	.0002
Dinner												
Fried cornmeal mush:			1.6	.2	14.0							.022
Cornmeal—dry weight	20	40		40.0								
Pork fat	14		2.9	1.4	44.6							
Cane sugar syrup:					.8							
Sugar	163		.2	11.1	91.2							
Water	16		9.9		10.0							
Sweet potato—raw	10					.031	.046	.647	.063	.073	.153	.0012
Cabbage—raw shredded						.007	.002	.039	.004	.005	.007	.0001
Cornmeal muffins (3 muffins)						.019	.048	.161	.051	.138	.108	.0009
Sugar												
Coffee												

TABLE I (continued)

Food	Weight	Protein	Fat	Carbo-hydrate	Ca	Mg	K	Na	P	Cl	S	Fe
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
Supper												
Fried cornmeal mush:†												
Cornmeal—dry weight	20	1.6	.2	15.9	.004	.017	.043	.008	.038	.029	.022	.0002
Pork fat	40		40.0									
Cane sugar syrup:‡												
Sugar	14			14.0								
Water												
Spinach	60	1.2	.1	1.9	.010	.022	.046	.075	.041	.044	.023	.0015
Cornmeal muffins (3 muffins)		9.9	11.1	91.2	.019	.048	.161	.051	.138	.108	.148	.0009
Sugar	15			15.0								
Coffee												
		40.5	115.3	448.2	.147	.268	1.362	.318	.660	.602	.608	.0062

Calories from protein............ 162.0
Calories from fat................ 1037.7
Calories from carbohydrate....... 1792.8
Total calories................ 2992.5

Sources of figures: Sherman, H. C. "Chemistry of Food and Nutrition," 4th ed.

Materials	Weight	Protein	Fat	Carbo-hydrate	Ca	Mg	K	Na	P	Cl	S	Fe
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
Cornmeal	138	11.4	1.7	109.7	.0248	.1150	.2939	.0538	.2622	.2014	.1531	.0012
White flour	165	18.8	1.6	123.9	.0330	.0297	.1907	.0090	.1518	.1221	.2920	.0016
Pork fat	30		30.0									
Sugar	40			40.0								
Baking powder	5											
Salt	4											
Total—9 muffins		30.2	33.3	273.6	.0578	.1456	.4816	.1528	.4140	.3235	.4451	.0028
1 muffin		3.3	3.7	30.4	.0064	.0161	.0538	.0169	.0460	.0359	.0494	.0003

* Recipe for cornmeal muffins.
† Cornmeal mush. Cornmeal weighed dry, cooked in boiling salted water, allowed to cool, sliced, fried in pork fat and served with cane sugar syrup.
‡ Cane sugar syrup. Sugar weighed, water added, cooked to make syrup.

Each patient began to improve within twelve to twenty-four hours after the first administration of ventriculin and the glossitis and stomatitis, which had become worse on the basic diet, healed so rapidly that within seventy-two hours from the beginning of the administration of ventriculin, the oral lesions had disappeared. It was noteworthy that the patient's desire for food increased soon after the ventriculin was given. Since none of these patients had the neurological manifestations of pellagra, the therapeutic effect of ventriculin on the central nervous system involvement could not be determined. The skin lesions had begun to improve on the basic diet (an observation which has been pointed out previously (8)) before ventriculin treatment was initiated so it was impossible to determine whether or not ventriculin aided in healing them under these conditions.

Since ventriculin is efficacious in the treatment of pernicious anemia and pellagra and since both conditions have certain clinical similarities, one speculates as to whether or not the same substance in ventriculin cures both diseases. Castle (27) believes that pernicious anemia is a condition secondary to a lack of some unknown constituent in the patient's gastric juice. Spies and Payne (20) incubated the gastric juice of two acute pellagrins receiving a vitamin G-free diet (glucose and lactose) with beef and found that the mixture contained an antianemic substance capable of producing a remission in pernicious anemia. They suggested that, in the pathogenesis of the two conditions, the usual pellagrin develops his disease following inadequate ingestion of food and that the pernicious anemia patient has a predisposing gastric dysfunction which prevents the proper assimilation of his food.

Ventriculin must be regarded as a very complex substance. It cures or prevents the manifestations of vitamin G deficiency in white rats and prevents the development of anemia in dogs receiving a pellagra-producing diet over a long period of time (21, 22). It must be realized, furthermore, that the knowledge of pellagra and pernicious anemia is far from complete and, though there may be some possible relationship in the pathogenesis of the two conditions, speculation at this time is not justifiable. This is particularly pertinent in that the chemical nature of the antianemic and antipellagric factors is not yet understood.

III. Parenteral liver extract as an aid in treatment

A short time ago Goldberger and Sebrell (23) found that liver extract fed to dogs in large amounts either prevented or retarded the development of black tongue. Boggs and Padget (7) stressed the curative value of liver (not liver extract) in pellagra and Smith and Ruffin (9) and Spies (8) have found that large amounts of liver extract by mouth are efficacious in treating the disease. Ramsdell and Magness (24), in a preliminary study on the effects of intramuscular liver extract on the course of pellagrins, gave small (2 cc.) daily doses and at the same time allowed pa-

tients a highly nutritious diet. They felt that the intramuscular administration of liver extract resulted in clinical improvement, but were frank to state that the patients were not given a deficient diet. Recently Ruffin and Smith (28) under more carefully controlled conditions observed no therapeutic effect when 5 cc. of parenteral liver extract were given. It has been recognized for a long time that the high mortality rate in severe pellagra is usually the result of central nervous system involvement, pernicious vomiting or an intractable diarrhea. As might be expected, many of these patients either refuse food or are unable to assimilate or absorb it and the disease progresses until death. Since the mortality rate is so high in the severely diseased pellagrin (54 to 69 per cent), it was decided to determine under controlled conditions whether or not parenteral liver extract is a valuable therapeutic agent capable of reducing the mortality rate of the disease.

Sixteen patients with the classical skin and oral lesions of pellagra were selected for this method of treatment. The results following the treatment of some of these patients have been reported in a preliminary communication (29). Each patient was placed on the unbalanced diet, low in so-called "vitamin G," at the time of admission and was not urged to eat more than he chose. After the stomatitis and glossitis had become definitely worse in each instance, 80 cc. of liver extract[a] were administered intravenously in four doses to each of ten patients during the subsequent twenty hours. This treatment was followed by the healing of the oral lesions after which no further injections of liver extract were given. Seven of the ten were then given a well balanced diet. The remaining three were still offered the basic diet until each patient relapsed and had a recurrence of mild but definite oral lesions of pellagra. Again these patients were given liver extract in the same manner as described before and the pellagrous condition subsided. Each of the remaining six of the sixteen pellagrins was given three intramuscular injections of liver extract totaling from 24 cc. to 30 cc. within a period of thirty hours.

No untoward reactions occurred from the intravenous or intramuscular injections of liver extract and twelve to twenty-four hours later the patients were improved. In each instance the tongue and oral mucous membranes, which had become progressively worse on the basic diet, appeared less red and swollen within twenty-four hours after the first injection. Seventy-two hours after the liver therapy the lesions had healed and were grayish pink in color instead of the fiery red observed prior to treatment. The three patients who remained on the restricted diet again developed lesions of the tongue and mucous membranes within ten days, but dermatitis did not reappear during this short time. These relapses were likewise successfully treated by means of intravenous liver extract.

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[a] Furnished through the courtesy of Dr. E. A. Sharp, Parke, Davis & Co.

The patient's desire for food increased following this therapy. In these patients the skin lesions improved rapidly. Under the conditions of this experiment, the changes in the dermatitis cannot be attributed to a specific action of liver extract. Since the patients in this study had no mental symptoms, the effect of parenteral liver extract on neurological manifestations could not be determined.

The present study has demonstrated that either the intravenous or the intramuscular administration of large amounts of potent liver extract causes a remission of the oral manifestations of pellagra. It has been previously pointed out (10) that the oral lesions, and not the dermatological ones, should be used for prognosis and the testing of materials for therapeutic efficacy. It is already known that an adequate diet usually prevents the development of pellagra and oftentimes cures it when it has begun (3), but the mortality rate in institutions admitting severe pellagrins is from fifty-four (25) to sixty-nine (7) per cent, even when the patients are offered a highly nutritious diet. It was realized some time ago that this high mortality rate was due in part to the patient's inability to eat and therefore efforts were made to develop a parenteral therapeutic agent (26). While an adequate diet is usually curative for patients with mild pellagra, it seems on theoretical grounds that their convalescence might be shortened by either intravenous or intramuscular injections of potent liver extract. It should be especially useful as an aid in treating the severe pellagrins who cannot or will not eat sufficient quantities of food.

SUMMARY AND CONCLUSIONS

1. It has been shown in the present experiments that the oral lesions of pellagra heal rapidly while the patient's diet is restricted to large amounts of either autoclaved yeast or ventriculin.

2. These findings suggest that there may be some etiological relationship between pellagra and pernicious anemia.

3. The evidence is too meager at the present time to justify the designation of vitamin G as the specific substance capable of curing pellagra.

4. It has also been shown in the present experiment that the oral manifestations of sixteen pellagrins responded dramatically following the parenteral administration of massive doses of liver extract.

5. The use of large doses of liver extract is suggested, either intravenously or intramuscularly whenever a severe pellagrin has difficulty in ingesting or assimilating sufficient quantities of a highly nutritious diet.

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THE RADIATION OF HEAT FROM THE HUMAN BODY. IV. THE EMISSION, REFLECTION, AND TRANSMISSION OF INFRA-RED RADIATION BY THE HUMAN SKIN

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INTRODUCTION

Although the absorption and reflection by skin of ultra-violet and visible radiation has been studied extensively, beginning with the spectrographic observations of Hasselbalch (1) in 1911, there has been relatively little investigation of these properties of the skin in respect to the infra-red region of the spectrum. This is especially true of the spectroscopic study of the skin in relation to infra-red radiation, as most of the investigations reported in the literature have been carried out by means of filters.

Our interest in the long wave length reflecting and transmitting powers of the skin lies in the relation of this form of radiation to the heat regulating mechanism of the body. As is well known, the heat produced by the human body is dissipated to the environment by evaporation, conduction, convection, and by radiation. Much evidence has accumulated in the course of studies of skin temperature (Aldrich (2), Cobet and Bramigk (3), Hardy (4)), not only that the radiation loss occurs entirely within the infra-red region of the spectrum, as would be expected, but that within the range of effective radiation the skin obeys the laws of black-body emission. As a consequence, one would expect to find no reflection and, conversely, complete absorption of radiant energy within this range of the spectrum by the outermost layers of the skin, since the radiating character of a body depends on the nature of its surface.

Sonne (5) carried out some experiments on reflection by skin-like surfaces of radiation in the visible and in the so-called "inner" and "outer" infra-red regions of the spectrum, using filters, and found 35 per cent reflection in the visible and "inner" infra-red and no reflection in the "outer" infra-red. By means of needle thermocouples he also observed the transmission through living skin and reports a gradient of temperature from without inward with a maximum 0.5 cm. below the skin surface in the case of visible and "inner" infra-red, while the "outer" infra-red showed little power of penetration, the maximum heating effect occurring at the surface. Loewy and Dorno (6) report similar penetration by the visible and shorter waved infra-red and similar impenetrability of the

longer waved infra-red.   Bachem and Reed (7), in the course of experiments on transmission through various layers of skin of light, chiefly in the ultra-violet and visible but extended to include the infra-red out to 1.4 μ, found increasing absorption in the outer layers with increasing wave length.   Danforth (8) and Cartwright (9) have studied spectroscopically the transmission of visible and infra-red through the human cheek and their curve shows penetration only between 0.54 μ and 1.5 μ with a peak at 1.15 μ.

As may readily be appreciated the above mentioned experiments by previous workers lend support to the theory that the skin, although it reflects and transmits considerable visible energy and energy in the infrared, is virtually a perfect absorber in the region beyond 3 μ, which Plank's equation gives as the range of the spectrum in which a body at the temperature of human skin radiates energy.   The evidence, however, is fragmentary and there do not exist, to our knowledge, any comprehensive spectroscopic studies of the absorptive and emissive properties of the skin throughout the region of the infra-red spectrum in which the body radiates energy.   In addition to the experiments already mentioned, however, which have bearing on the probable black-body character of the skin, are the experiments on transmission of Pauli and Ivancevic (10), Gaertner (11), and Pearson and Norris (12) and the measurements of the emission spectrum of skin recently reported by Wright and Telkes (13).

The following experiments fall into three groups: (1) Experiments on the emission spectrum of the skin compared to the emission spectrum of an experimental black-body.   (2) Experiments on the infra-red reflection spectrum of skin.   (3) Experiments on the infra-red transmission (absorption) spectrum of skin.   In the first two groups living human skin has been used as the subject of the experiments.   In the third group, skin from freshly amputated surgical specimens and epidermal layers of skin, obtained by blistering with cantharides plasters, were used.

METHOD

A reflecting infra-red prism spectrometer equipped with a rock salt prism of refracting angle 60°, Wadsworth mounting, and adjustable slits was used in conjunction with a vacuum thermocouple connected to a Leeds and Northrup high sensitivity galvanometer.   A schematic drawing of the apparatus is shown in Figure 1 in which S_1 and S_2 represent the slits of the spectrometer, P the prism, M_1, M_2, M_3, plane mirrors, C_1, C_2, C_3, concave spherical mirrors, O the axis of rotation of prism table, and T the thermocouple.   Amplification of the galvanometer deflections was secured by a thermo-element device and a second galvanometer but the sensitivity of the apparatus without amplification was actually sufficient and the amplifier was used only in some of the experiments upon skin emission.   A

Nernst glower was used as the energy source for the experiments dealing with reflection and transmission. Calibration of the spectrometer was carried out by the determination of eight points, viz., quartz reflection peak at 12.5 μ, quartz reflection peak at 8.9 μ, CO_2 emission peak at 4.35 μ, water absorption band at 2 μ, mercury arc infra-red line at 1 μ, visible red at 0.75 μ, visible yellow at 0.58 μ, and visible green at 0.54 μ, and a calibration curve was plotted through these points. The mounting of the skin specimens, in relation to the energy source and the entrance slit of the spectrometer, in the experiments dealing with reflection and transmission, will be described in the sections devoted to these experiments. The slits used in the experiments were for the most part fairly wide, 0.3–0.4 mm., but in some regions of the spectrum when working with very thin layers of skin in the transmission experiments, very narrow slits, 0.05 mm., were used. For the emission experiments, because of the low intensity, very wide slits, 1 mm. had to be used. The data given below are without slit width corrections.
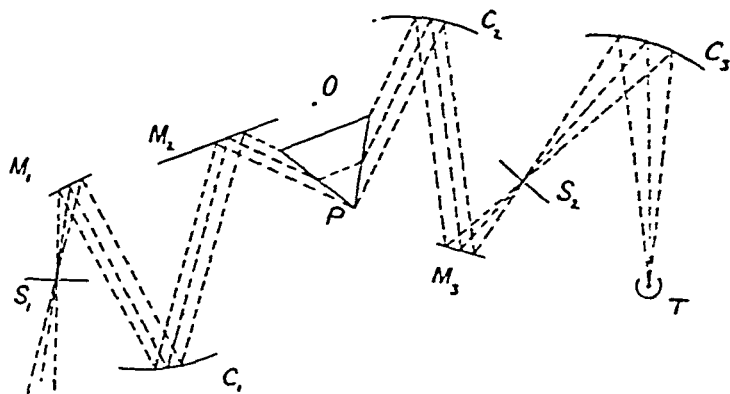
FIG. 1.   SCHEMATIC DIAGRAM OF SPECTROMETER

EXPERIMENTAL

1. EMISSION SPECTRUM OF HUMAN SKIN

The subject's finger was held immediately in front of the spectrometer slit, and readings of the galvanometer deflections were made at 5' intervals on the spectrometer scale. Between each reading the finger was removed and the galvanometer allowed to return to the zero point. Similar observations were then made on a Leslie cube, the cone of the cube being applied to the window in the spectrometer housing just before the slit. The Leslie cube was used as an experimental black-body and was maintained at a temperature as close to that of the subject's skin as possible throughout the period of observation. The measurements of skin temperature were

made with the radiometer previously described. The results of one such experiment are given in the form of a curve (Fig. 2), in which wave lengths are plotted as abscissae and galvanometer deflections as ordinates. Amplification of the galvanometer deflections was employed. Other similar experiments, with and without amplification, gave similar results and are not reproduced here. Examination of the figures reveals that the skin emission curve and the Leslie cube emission curve are nearly superimposable. The latter has been shown by Hardy (4) to agree within 1 per cent with the theoretical curve for black-body emission derived from Planck's formula. The absorption bands present in the curve are of no significance as they are due to the protective lacquer coatings on the collimating mirrors of the spectrometer. The presence of the band at 8 μ, however, accounts for the apparent shifting of the maximum from its theoretical position at 9 μ to its observed position at 6 μ. The location of the cutoff in the near infra-red, as may be seen, lies somewhere between 2 μ and 3.5 μ, its exact location probably nearer 3.5 μ since the slit widths were necessarily large and the data are plotted as observed, that is, without slit width corrections. Essentially the same results were obtained by Wright and Telkes (13).
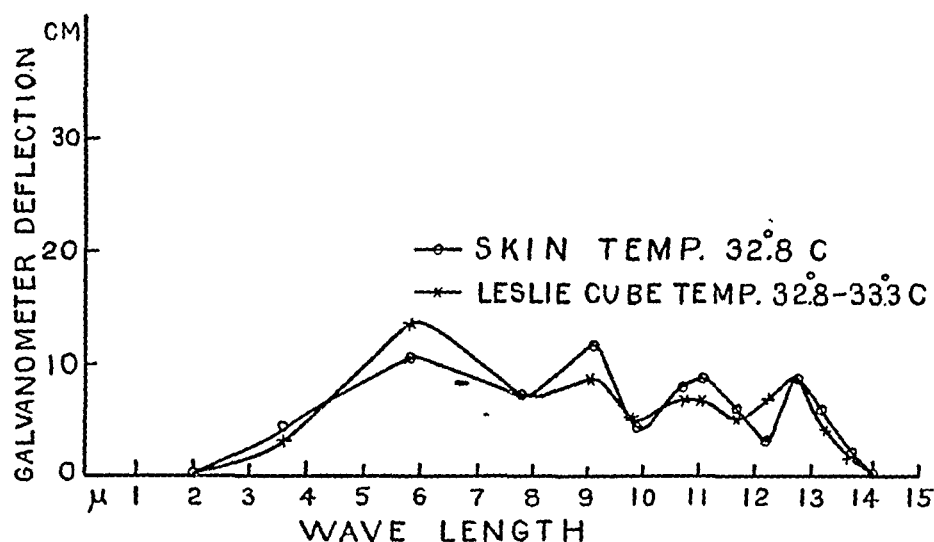
FIG. 2. EMISSION CURVE OF HUMAN SKIN AND OF LESLIE CUBE

2. INFRA-RED REFLECTION SPECTRUM OF HUMAN SKIN

The study of reflection obviously offers difficulties because of the diffuse character of reflection from the skin. The method chosen was to compare the reflection from skin with that from a substance of known reflecting power. To do this it is, of course, necessary to use a substance which reflects diffusely and the total reflection of which over the entire hemisphere is known. If the surface chosen obeys Lambert's law of dif-

fuse reflection to the same extent as the skin it is only necessary to make the comparison at a single angle of reflection. The total reflection of the skin will then be given by the expression $(I_\theta/I_\theta') \times R' = R$ where I_θ is the energy reflected at angle θ from skin, I_θ' the energy reflected at angle θ from the comparison substance, R' the total reflection from the comparison substance and R the total reflection from the skin. The substance used for comparison in the following experiments was a scraped block of magnesium carbonate. The total reflecting power of this substance has been determined by Coblentz (14) at five wave lengths between $0.6\,\mu$ and $24.0\,\mu$.
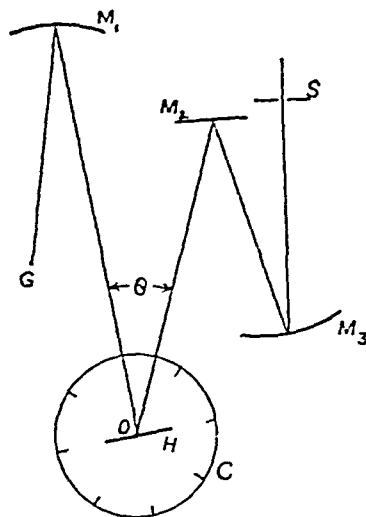
FIG. 3.   DIAGRAM OF EXTERNAL OPTICAL SYSTEM FOR REFLECTION AND TRANSMISSION EXPERIMENTS

To test Lambert's law an apparatus was devised, after the manner of Hutchins (15), to serve as an external optical system. A schematic diagram of the system is shown in Figure 3, in which S represents the entrance slit of the spectrometer, G the Nernst glower, H a brass holder with rectangular aperture against which the specimen for examination is applied, M_1 and M_3 concave mirrors, and M_2 a plane mirror. The holder H is mounted so that it may rotate with respect to a graduated circle C but so that it is fixed with respect to M_1 and G and that the incident beam is normal to the reflecting surface. G, M, and H thus rotate together about the point O. With this arrangement, when the image of G is focussed at O, if M_2 and M_3 are properly adjusted, the light diffusely reflected from O at any angle θ always falls upon the slit S. Measurements were made of the energy reflected at various angles from the normal from each of the substances while the spectrometer was set at $1.3\,\mu$ and also with the spectrometer set at $3.6\,\mu$. The results are shown in Table I where θ denotes

the angle of reflection, I^{θ} the galvanometer deflection (intensity of reflected beam) for skin at angle θ, I_{θ}' the galvanometer deflection for magnesium carbonate at angle θ. The ratios $I_{\theta}/\cos\theta$ and $I_{\theta}'/\cos\theta$ should in each case be constant according to Lambert's law. The results show that

TABLE I

Tests of Lambert's Law of diffuse reflection for skin and magnesium carbonate surfaces

Wave length	θ	$\cos\theta$	Skin		Magnesium carbonate	
			I_{θ}	$I_{\theta}/\cos\theta$	I_{θ}'	$I_{\theta}'/\cos\theta$
μ	*degrees*		*cm.*		*cm.*	
1.3	20	.940	2.2	2.34	11.5	12.2
	30	.866	1.9	2.19	10.3	11.9
	40	.766	1.9	2.49	9.8	12.8
	50	.643	1.5	2.33	7.8	12.1
	60	.500	1.5	3.00	6.6	13.2
3.6	20	.940	3.0	3.19	8.2	8.73
	30	.866	2.6	3.00	7.8	9.00
	40	.766	2.3	3.00	7.6	9.90
	50	.643	1.8	2.80	5.5	8.55
	60	.500	1.5	3.00	4.2	8.40

in fact the ratio is very nearly constant for each of the substances. In general, the findings indicate that the skin and the magnesium carbonate surface both follow Lambert's law sufficiently closely to enable one to use the latter as a standard for determining the reflectivity of skin.
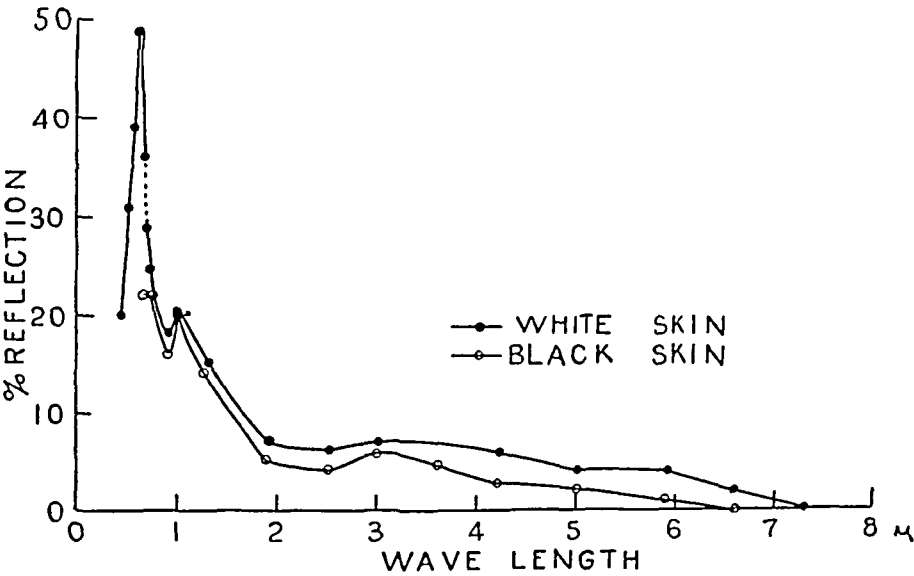
FIG. 4. REFLECTION OF WHITE AND OF NEGRO SKIN

Figure 4 shows the percentage reflection from white and negro skin in the infra-red, as measured in the manner outlined above, using the same optical arrangement as that used in the tests of Lambert's law. The angle θ was maintained at 20° and direct comparisons between the skin and the block of magnesium carbonate were made at each spectrometer setting. The skin of the volar surface of the forearm was used. On white skin measurements were also made in the visible, using a Koenig-Martens spectrophotometer instead of the infra-red spectrometer. The dotted line indicates the discrepancy, 7 per cent, between the spectrometer measurements and the spectrophotometer measurement at 0.7μ. The discrepancy is undoubtedly due to inaccuracy of the spectrometer method in this region of short wave lengths, since the energy of the Nernst glower source is low here and the galvanometer deflections are correspondingly small with large percentage error. It is for this reason that the spectrophotometer was used throughout the visible range.

The significant findings on examination of the curves are that there is a sharp falling off in reflecting power at about 2μ, in the region of the cut-off in the near infra-red of the skin emission curve, and that beyond this region the reflection is only in the neighborhood of 5 per cent. It is also significant that the amount of reflection in the infra-red is about the same for negro skin as it is for white skin.

3. TRANSMISSION SPECTRUM OF HUMAN SKIN

In the study of transmission it was obviously necessary to use dead skin unless animals were to be employed. Accordingly, fragments of human skin were obtained from specimens from surgical amputations and kept moist with normal saline solution up to and during the time of making the observations. This was always on the day on which the specimen had been removed from the patient. The pieces of skin thus obtained by trimming with a scissors or stripping with a knife from the underlying subcutaneous adipose tissue of the amputated breast or leg were quite thick and required further shaving down to eliminate as much of the adherent subcutaneous tissue as possible. This was attempted by means of a freezing microtome but, with the size of the skin specimens required, it was found impossible; therefore, instead of shaving with a microtome knife, after freezing on the stage of the microtome, the specimens were filed down to the desired thickness with a hand file. In all specimens so treated, it was found later by cross sectioning and appropriate staining, that all the layers of skin were left intact and that a thin layer of subcutaneous tissue also remained. For studying the transmission of the epidermal layers of skin alone, specimens were obtained by means of blistering the skin of living subjects with cantharides plasters and, about twelve hours after application of the plaster, removing the layers of epidermis separated by this process. These speci-

mens were then similarly kept moist with saline solution and examined on the days of their removal from the subjects. They were also constantly kept moist with the saline solution by sponging during the period of spectroscopic examination. The small amount of water on the tissue during the time of measurement could not have represented a film of greater thickness than 0.05 mm. Such a thickness transmits the infra-red readily.

The external optical arrangement for most of the transmission experiments was similar to that used in the reflection experiments, the only differences being that the angle θ, Figure 3, was set at 180°, for the direct transmission, and that a new holder was substituted which was so constructed that the skin could be stretched over a rectangular hole, 2×1 cm., in the holder. The holder was set, similarly to the one used in the reflection experiments, so that the plane of the surface of the skin was normal to the incident beam. By this arrangement the effect of scattering could be studied as well as the direct transmission, since by changing θ from 180° the energy of the radiation emerging at various angles from the direct path could be measured. The transmission curves shown in the accompanying figures, however, are not corrected for scattering but represent the ratio of the deflection caused by the directly transmitted beam to the deflection caused by the incident beam, expressed in percentage. The observation on the energy of the incident beam at each point in the spectrum was not made immediately after each observation on the skin since it was necessary to dismount the skin from the holder. The entire spectrum was therefore explored first with the skin in place and then with the empty holder, or vice-versa. The transmission values are calculated from the ratio of the galvanometer deflections produced by the energy transmitted *directly* through the skin to the deflections produced by the energy of the direct beam with the skin removed.

A. Transmission through entire thickness of skin

Only one of the experiments on transmission through the entire thickness of the skin is reproduced here (Fig. 5) since all show similar results, that is, practically complete absorption. In some of the experiments instead of using the external optical system described above, the number of mirrors was reduced to one and the skin specimen was held about two centimeters before the spectrometer slit, to determine whether there was a significant loss due to the number of mirrors; but the results were essentially the same. The curve shown is for a piece of skin which, measured *after* fixation and staining, was found to be 1 mm. thick, about half of its thickness being actual skin and the rest adherent subcutaneous tissue. The slit widths necessary for producing any significant deflection when this very opaque, thick skin was interposed were so large (1 mm.) that the comparative measurements on the incident beam gave too large deflections to be

read directly and the transmission ratios had to be interpolated, through the major portion of the spectral region concerned, by further comparison with reduced slit widths. Therefore, the recorded transmission values cannot be considered more accurate than ± 2 per cent in absolute value, but can be considered as giving the order of magnitude of the percentage transmission.
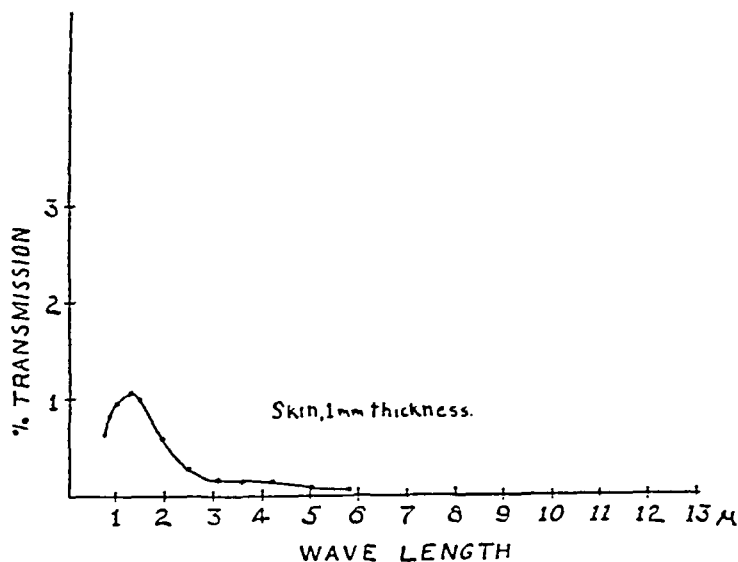
FIG. 5. TRANSMISSION THROUGH SKIN AND SUBCUTANEOUS TISSUE, THICKNESS 1 MM.

These results, of extremely low penetrability even in the near infra-red, do not agree with the findings of most previous workers. Danforth (8) and Cartwright (9) obtained appreciable amounts of transmission out to about $1.5\,\mu$ through thicknesses of skin and subcutaneous tissue greater than those studied by us. It is possible that the fact that we were using skin that was dead and had been frozen while their work was carried out on the living tissues accounts for the discrepancy, although the work of Bachem and Reed (16) has shown that in the ultra-violet and visible there is little difference between the penetration through living and through dead tissue. The results of Pauli and Ivancevic (10) and of Gaertner (11) are also in apparent disagreement with ours but their methods, which involve placing the specimen close to the receiving thermo-element, sometimes in front of and sometimes behind the exit slit, are open to the objection that re-radiation is not eliminated. Unfortunately, we could not study the effect of scattering by the thick pieces of skin but our observations on scattering by the thinner epidermal pieces, to be recounted below, are in total disagreement with the observations on scattering of Pauli and Ivancevic.

They found large amounts of energy at all angles of emergence, contrary to our own findings, but did not distinguish between actually transmitted scattered radiation and re-radiation due to heating of the skin, a distinction which can only be made if the specimen is placed in front of the dispersing prism. In agreement with our results are those of Aldrich (2) who measured the total transmission through skin 2 mm. thick and found it to be negligible in amount.

B. Transmission through epidermis

The transmission curves of several pieces of epidermis obtained by blister from the volar surface of the forearm of each of three individuals are shown graphically in Figure 6. The specimens were sectioned and

FIG. 6. TRANSMISSION THROUGH EPIDERMIS

Specimen 1. White corneum and Malpighium, thickness 0.12 mm. Specimen 2a. White corneum, thickness 0.03 mm. Specimen 2b. White Malpighium, thickness 0.08 mm. Specimen 3. Negro corneum, thickness 0.14 mm.

stained with hematoxylin and eosin, after the experiments, to establish which layers of epidermis were included in each piece of skin studied. Specimen 1 is skin from a white man and includes the corneum and a large part, if not all, of the Malpighian layer, thickness 0.12 mm. Specimen 2

is also white skin but in this case separation between the corneal layer (2a), thickness 0.03 mm., and the Malpighian layer (2b), thickness 0.08 mm., was fortuitously obtained, the corneum having at first adhered to the cantharides plaster and the Malpighian layer to the raw surface of the arm. Specimen 3 is from the arm of a very dark negress and consists of the corneal layer from which most of the Malpighian layer was apparently lost in removal. Its thickness, 0.14 mm., is considerably greater than that of the corneum of white skin, in fact it is approximately that of the corneal and Malpighian layers of white skin taken together. Its opacity also is greater than that of the white corneum, but is slightly less, in the longer wave lengths at least, than that of the white Malpighian layer. It is a question whether the intermediate position of this curve is due entirely to the thickness of the negro corneum or whether it is also partly due to the presence of a few scattered cells of the underlying Malpighian layer. Pigment is, of course, also present, but the amount is not large in the outer layers which are under consideration.

On studying the curves it is seen that in the near infra-red there is considerable transmission through both corneum and Malpighian layer but that beyond $3\,\mu$, while the corneum still transmits a large percentage of energy, the transmission through the Malpighian layer alone and through thicknesses containing both layers falls off markedly. It will also be observed that in Curves 2a, 2b, and 3 there are well marked absorption bands and that the most prominent of these is present in all three of the curves. This band is not found in Curve 1 because the points were taken too far apart to resolve it.

The results of the experiments on transmission through epidermis agree in general with the findings of Pearson and Norris (12) who made similar studies, but only to $5\,\mu$. The greater percentage transmission found by them may be accounted for partly by the fact that the layer of skin used was thinner than any studied by us, but is probably also due to the fact that, in the effort to correct for scattering, they placed the specimen behind the exit slit and in close proximity to the thermopile, thus introducing the error of re-radiation.

The effect of scattering

The effect of scattering was studied in the transmission experiments on the thin layers (epidermis). The skin holder was mounted, as previously mentioned, on the same graduated circle used for the reflection experiments. In studying the direct transmission the holder H (Fig. 3) was maintained in such position that θ was $180°$. The energy of scattered beams emerging at various angles from the direct beam could now be directly measured by rotating to any desired angles away from $180°$. The results of such experiments, made at several different points in the

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CONCLUSIONS

The energy distribution curve of the radiation of the normal human skin has been found to correspond with that which would be expected of a physical black-body of the same temperature. The low reflecting and transmitting power of the skin for radiation in the region of the spectrum in which the skin radiates is further evidence in support of this conclusion. The visible color of the skin exerts no influence on its absorbing power in the infra-red. The absorption and emission of infra-red radiation occurs in the outer layers of the skin. The infra-red transmission spectrum of skin has a characteristic fine structure which may prove to be of physiological interest.

SUMMARY

1. The emission spectrum of human skin has been studied and found to be essentially that of a black-body radiator.

2. The infra-red transmission and reflection spectra are such as would be expected of a black-body radiator.

3. The infra-red transmission spectrum of skin epidermis has been found to have a characteristic structure. The most prominent absorption bands are situated in regions where there are known to be strong bands due to C–H, N–H, and O–H linkages.

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LOCALIZATION OF PAIN ACCOMPANYING FARADIC EXCITATION OF STOMACH AND DUODENUM IN HEALTHY INDIVIDUALS [1]

By EDWARD A. BOYDEN and LEO G. RIGLER

(*From the Departments of Anatomy and Radiology, University of Minnesota, Minneapolis*)

The observations recorded in this article were originally by-products of a group of experiments designed to test whether or not the human gall-bladder is subject to inhibitory reflexes originating in the gastro-intestinal tract (1). Subsequently, these experiments were repeated and elaborated in the belief that pain originating from ring contraction of the gut might be more specifically localized than sensations arising from inflamed or distended surfaces of the hollow viscera, and so throw additional light on the baffling problem of splanchnic pain.

METHODS

The method of investigation consisted of sending an induction current through a Rehfuss tube, the metal end of which had been converted into an electrode and swallowed to the desired depth. The second electrode was made of a moist felt pad sewed to a copper screen and applied to the arm or leg. The subjects chosen for experimentation were eleven volunteer medical students in the University of Minnesota, who could be depended upon for intelligent and trustworthy cooperation.

The strength of current employed, as measured by the position of the secondary coil over the core of a Harvard inductorium, was similar to that used in ordinary physiological experiments. When the induction coil was attached to two dry cells, the minimal stimulus required to produce visceral sensation varied with the individual but ranged from Position $6\frac{1}{2}$ to Position 5, i.e., with the secondary coil from 1 to $2\frac{1}{2}$ centimeters over the end of the core. The maximum stimulus used (Position 4) was of a strength which was unbearable when applied to the lips, but still tolerated by the gut.

PRELIMINARY OBSERVATIONS

The effect of the current upon the stomach, as observed under the fluoroscope, was to induce a sphincteric contraction of the gut and then an

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PRELIMINARY OBSERVATIONS

The effect of the current upon the stomach, as observed under the fluoroscope, was to induce a sphincteric contraction of the gut and then an

increased peristalsis distal to the point of stimulation. Occasionally a whole segment of the gut would contract uniformly (see Figure 4 of article cited (1)). If the electrode was not in contact with the wall of the stomach but merely lay in its cavity, the effect of the current was much reduced. Once, in such a case, peristalsis already in progress was checked by the current. Occasionally, after prolonged experimentation, the subject failed to respond to the stimulus. This was attributed to mucus (afterwards regurgitated with the tube) which apparently collected in such quantities as to insulate the gut against the current; for when the position of the tube or patient was changed, the response to the current was restored.

The effect of the current upon the duodenum could not be ascertained because the barium passed through this portion of the gut so rapidly. It was presumed from animal experimentation, however, that the current caused ring contraction of the intestine also.

In both organs contraction of the visceral musculature was usually accompanied by some degree of abdominal rigidity, depending on the strength of the current. Sometimes this rigidity occurred when the current was too mild to induce any visceral sensation. Also it seemed to be more pronounced when the second electrode was fastened to the arm than when it was applied to the leg.

The nature of the sensation that accompanied contraction of the gut ranged from barely perceptible feelings of pressure, gnawing sensations and heart-burn, to dull and severe colicky pain. Frequently there was a "throbbing" sensation which apparently synchronized with the alternation of the current. When a mild current was employed, one or more seconds usually elapsed before visceral sensations were felt. Then the pain increased gradually to a climax. In the case of very strong currents, causing spastic contraction of the gut, the pain was immediate.

Localization of these sensations was characterized by two general features: (1) the depth of the sensation (it seeming to come from well beneath the abdominal wall); (2) the definiteness with which it could be located in the upper quadrants of the abdomen (the subject always pointing to the spot with one finger).

The localization of pain

1. *Posture constant.* The first group of experiments (Figure 1) represent a summary of observations made upon one student on six different days scattered over a period of several months. Figure 2 records incidental observations made upon five other students in connection with gall-bladder experiments. In each case the subject was lying prone on the x-ray table—the position in which stomach and duodenum approach nearest to the x-ray plate. The circles in each figure indicate the position of the electrode in the gut as determined by x-ray films taken immediately

before the current was applied. The dots indicate the area on the abdominal wall to which the subject pointed immediately after the current was interrupted.

A cursory examination of these figures shows two apparently contradictory features: a tendency for the pain areas to follow the course of the
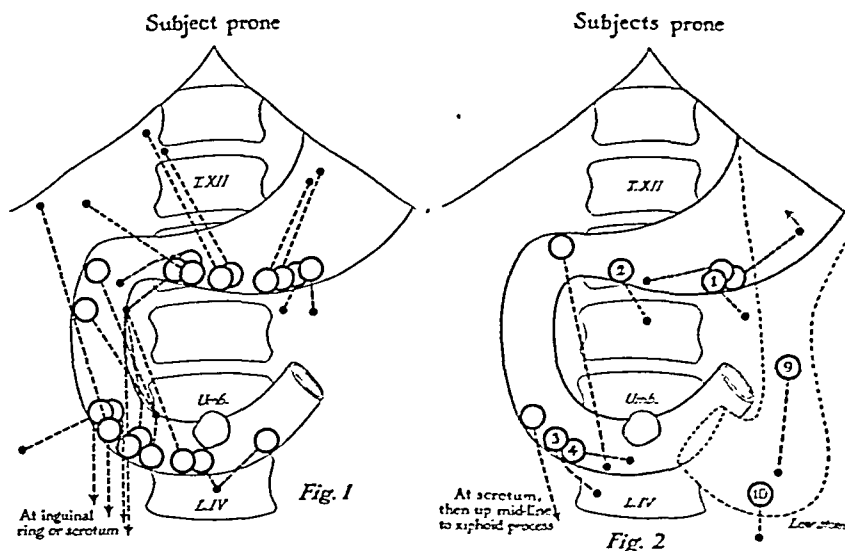
FIG. 1. ASSEMBLY DRAWING SHOWING LOCALIZATION OF PAIN IN SAME SUBJECT ON SIX DIFFERENT DAYS (SERIES II–VII, CASE E. F. M.)

In each experiment faradic stimulation of stomach or duodenum was applied for a period of 10 seconds. *Circles* indicate position of electrode in gut as determined by x-ray films; *dots,* the position on abdominal wall pointed to by subject as site of pain. For other details, see Figures 3 to 6.

FIG. 2. LOCALIZATION OF PAIN IN FIVE OTHER NORMAL SUBJECTS

Same technique employed as before. 1 to 4, four successive readings made from one subject, Case A. M. L. (March 8, 1933): *1,* 9:50 a.m., weak current, initial pressure sensation suddenly changing to "feeling as if a bubble had burst" or as if subject had been "hit with a blow"; *2,* 10:16, strong current, sharp colic increasing in intensity, "pretty bad"; *3,* 10:47, strong current, "extreme colic"; *4,* 11:02, moderate current, dull ache increasing to sharp colic. 9 and 10, two readings from Case D. S. F. (February 27, 1933): *9,* 9:51 a.m., weak current, feeling of pressure; *10,* 10:20, strong current, dull pain.

stomach and duodenum—so that if certain dots were selected they would outline approximately the position of the intestinal tube; and a certain aberrancy whereby pain originating in the stomach is sometimes referred to the left or to the right border of the ribs instead of to the overlying region, while pain from the right upper duodenal flexure may be projected downward to the right side of the umbilicus; or pain from the right lower

duodenal flexure may appear in the right epigastrium or at the inguinal region.[2]

A good illustration of the apparent tendency of gastroduodenal pain to follow the course of the electrode is shown in Experiments 1 to 4 (Figure 2)—four readings from the same individual (A. M. L.) taken at 15 to 30 minute intervals. Even more striking is the case of a student with a low-lying stomach (Figure 2) that projected an inch or more below the umbilicus when the subject was prone and the stomach empty.[3] In this subject (D. S. F.) dull pain induced by stimulation of the upper limb of the stomach was not localized in the left epigastrium, but in the left umbilical zone (Experiment 9, Figure 2); and when, fifteen minutes later, the electrode was swallowed another two inches, to the bottom of the greater curvature, the pain area descended with it (Experiment 10).[4]

Observations such as these, seemed at first to render it unlikely that we were dealing with pain that was being referred from the viscera to the abdominal wall, because no matter what the position of this ptotic stomach was, its nerve supply should be the same as that of any other stomach, and so the pain should have been referred to zones of the 6th to 9th thoracic nerves. Instead it was localized in the territory supplied by the 11th thoracic nerve. This case seemed to indicate, therefore, that we were dealing either with true visceral pain—i.e., with pain directly felt in the wall of the stomach—or else with excitation of the anterior parietal peritoneum—as recently predicated by Morley (3).

This author reported, for instance, that the site of deep tenderness in acute obstructive cholecystitis descended with increasing distention (and consequent elongation) of the biliary vesicle; and that the area of deep tenderness in ulcer patients followed the change in position of the stomach or duodenum. He interpreted such gastric pains as being due either to mechanical or chemical stimulation of the anterior parietal peritoneum, which was not felt in the peritoneum but was localized in the immediately overlying skin—a so-called peritoneo-cutaneous radiation.

2. *Effect of change in posture.* Impressed with Morley's account we undertook to see how change of position would affect the site of pain. Employing the same subject as before, it was soon noted that the pain area

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[2] In the latter case, the sensations were mostly " quiverings " in the territory of the cremasteric muscle and so may have been due to a spreading of the current to the ureter or internal spermatic vessels, which lie just deep to the thin posterior wall of the duodenum. This seems the more probable since the spermatic cord is not affected by experimental distention of this part of the duodenum (Fig. 11).

[3] This is an extreme type, apparently falling within the small group which Moody (2) describes as occurring in 3.2 per cent of normal male students (cf. Figure 7 of article cited).

[4] Roentgenograms showing the exact position of the electrodes in this case may be seen in Figure 12, Boyden and Rigler (1).

FIG. 3. GROUP OF EXPERIMENTS ILLUSTRATING EFFECT OF CHANGE OF POSTURE ON SITE OF PAIN. CASE E. F. M., SERIES IV (JANUARY 21, 1934)

Circles indicate position of electrode; *dots* indicate site of pain; *arabic numerals* within circles, the sequence of experiments: *1*, 12:02 p.m., weak current, "dull muscular pull"; *2*, 12:08, weak current, dull ache, stronger sensation than at 1; *3*, 12:11, strong current, pain more severe, but still dull; seemed to "flutter" with buzzer of induction apparatus and to "pull toward the back"; *4*, 12:24, strong current, sharp colic, vibrating but stationary; *5*, 12:38, moderate current, dull pain; *6*, 12:44, moderate current, dull ache in region of spermatic cord, "felt like cremasteric muscle"; *7*, 1:03, moderate current, sharp pain: "not crampy but like line of pain"; *8*, 1:08, moderate current, dull cramp; *9*, 1:16, moderate current, dull "spasm"; *11*, 1:25, moderate current, dull spasm: *12*, 1:30, moderate current, colicky pain; *13*, 1:35, moderate current, faint flutter.

FIG. 4. GROUP OF EXPERIMENTS EXTENDING OBSERVATIONS SHOWN IN FIGURE 3. (ORIGINALLY DESIGNED TO TEST EFFECT OF THORACIC NERVE BLOCK.) CASE E. F. M., SERIES VI (FEBRUARY 15, 1934)

Arabic numerals indicate sequence of experiments: *3*, 7:50 a.m., strong current, dull sensation not painful; *4b*, 7:55, weak current for 5 seconds only, painless "tugging" inside; *4c*, 7:57, weak current for usual 10 seconds, sharp pain starting slowly and increasing to steady colicky pain (felt after current stopped), followed by "fluttering in spermatic cord." 8:23 to 9:45 a.m., injections of 400 cc. of 1 per cent novocain deep in intercostal spaces 6 to 11, also along infracostal borders, also subcutaneously, in unsuccessful attempt to anesthetize whole anterior abdominal wall: subject subsequently found to be four times as resistant to novocain as average individual; *5a*, 9:48, strong current, deep pain getting worse; *5b*, 9:50, strong current, sharp pain "worse" than *5a*; *6*, 9:56, strong current, severe colicky pain (all that subject could stand); *7*, 10:02, moderate current, dull pain; *8a* (on abdomen), *b* (on right), *c* (on left), no feeling with strong current; *8d*, strong current, 10:12, dull sensation (plus twitching over left tensor fasciae latae); *8c*, 10:14, strong current, barely noticeable sensation. (The lessened pain noted in Experiments 7 to 10 may have been due to the accumulation of mucus in the stomach, or to general narcosis caused by large amount of novocain administered.)

frequently (but not always) shifted when the subject turned onto his side or back or stood erect. Thus starting with the patient supine (Experiment 9, Figure 3) a shift of the body onto the right side lowered the pain area (Experiment 11); a rolling over onto the abdomen still further lowered it (Experiment 12); and a standing posture swung the pain area to the midline, but not as far down as one would expect (Experiment 13). Similarly, shifting the subject from back to abdomen (with consequent lowering of the duodenum) shifted the area of localization from above to below the umbilicus (Experiments 2 to 4, Figure 3; Experiments 4 and 5, Figure 4). These cases illustrate the tendency of the pain to follow the change in position of the gut. In Figure 5, however, the opposite tendency is recorded. Here (Experiments 2 and 3) a change from prone to supine position shifted the pain from high up in the epigastrium to the umbilicus, just the reverse of the movement of the gut. Thus not all observations were consistent with Morley's theory. This caused us to test it by other methods.

3. *Experiments designed to test the rôle of the parietal peritoneum.* Believing, on *a priori* grounds, that the current was not strong enough to penetrate the hollow viscus and still stimulate nerve endings in the anterior parietal peritoneum (a distance of several inches from the electrode) deep manual pressure was exerted over the lower end of the duodenum. This should have increased the pain by bringing the parietal peritoneum nearer to the area of current density. Yet no such increase of pain was noted.

Again, peritoneal pain should have been in the nature of a sharp stitch and should always have appeared immediately over the electrode—witness the experiments dealing with direct mechanical irritation of the anterior peritoneum (Capps and Coleman (4)). Yet in twelve cases in which the electrode was in that part of the stomach that lies against the anterior wall —and with the patient prone—the sensations reported were not stitch-like pains but pressure or burning sensations, dull or colicky pain; nor were they localized accurately enough to meet the above requirements for stimulation of the parietal peritoneum.

Furthermore, if the current were spreading from the gut, its direction should have changed when the second electrode was moved—say from the left forearm to the right calf (e.g., Experiments 2c to 2e, Figure 6)—yet moving the second electrode did not change the site of the pain. Accordingly, it was concluded from these experiments that the current could not have directly stimulated the anterior peritoneum.

4. *Apparent conditioning of nervous pathways.* While the above experiments were being conducted, a subject was encountered in which no modification of external or internal conditions seemed to change the site of pain. Thus when the subject was shifted from a prone to supine position and back again (Experiments 2 to 6, Figure 7)—the electrode remaining
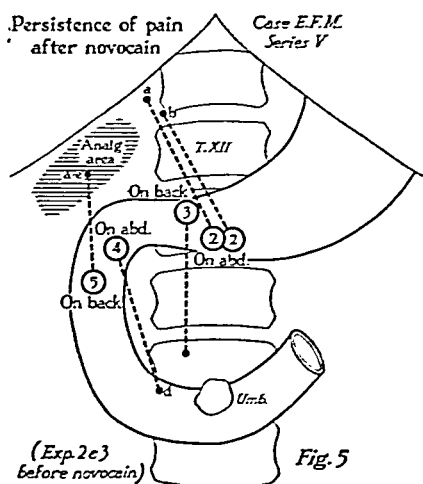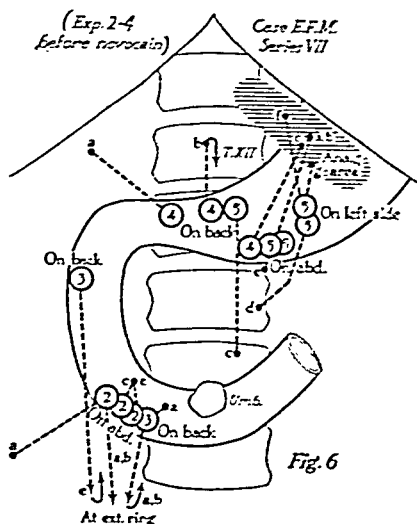
FIG. 5. GROUP OF EXPERIMENTS ILLUSTRATING PERSISTENCE OF VISCERAL PAIN UNDER AREA OF SKIN THAT HAD BEEN RENDERED ANALGESIC TO PIN PRICKS. CASE E. F. M., SERIES V (FEBRUARY 10, 1934)

Arabic numerals indicate sequence of experiments: *2a*, 7:47, weak current, gnawing sensation; *2b*, 7:49, strong current, gnawing sensation; *3*, 7:54, strong current, sharp, crampy pain, sort of "gone" feeling. 7:55 to 8:20, subcutaneous, wheal infiltration of ½ per cent novocain (and adrenalin) along right and left subcostal border; *4a–c*, not felt; *4d*, 8:32, moderate current, barely felt; *5a* and *b*, 8:35–:37, moderate current, dull tug, "pulling toward diaphragm"; *5c*, 8:39, weak current, dull sensation; *5d* and *e*, 8:41–:43, moderate current, dull sensation stronger than at *5c*, but not painful. (Note that in Experiments *5a–e*, a sensation was felt under an area analgesic to pin pricks.)

FIG. 6. GROUP OF EXPERIMENTS ILLUSTRATING EFFECT OF CHANGING POSITION OF SECOND ELECTRODE AND OF DEEP MANUAL PRESSURE OVER FIRST ELECTRODE. CASE E. F. M., SERIES VII (MARCH 3, 1934)

Arabic numerals indicate sequence of experiments: *2a*, 7:36 a.m., moderate current (2d electrode on left calf), dull vibrating pain barely felt, also flutter over right external ring; *2b*, 7:40, strong current, "cremasteric flutter"; *2c*, 7:43, moderate current (2d electrode on left forearm), dull pain; *2c*, 7:52, moderate current (2d electrode on right calf), dull vibrating sensation; *3a*, 8:01, moderate current (2d electrode on right calf), dull pain, more marked than in *2c*, also felt at external ring going deeper as it moves cephalad two inches; slight abdominal rigidity on right side only; *3b*, 8:03, moderate current, sensation in spermatic cord region moving up as before; deep pressure on abdominal wall over duodenal electrode caused no change in intensity of spermatic pain nor was any pain noted at umbilical region as before; *3c*, 8:10, moderate current (2d electrode on left forearm), dull pain starting at external ring and going deeper as it moved cephalad two inches (no other abdominal pain); *4a*, 8:18, moderate current, dull pulsating sensation; *4b*, 8:20, moderate current, deep, dull vibrating sensation which traveled downward; *4c*, 8:23, strong current, dull vibrating sensation. 8:30 to 9:10, subcutaneous infiltration of novocain along left subcostal border; *5a* and *b*, 9:15–:17, strong current, deep dull sensation same as in *4c*, appearing under area of skin analgesic to pin pricks; *5c*, 9:19, very strong current, dull sensation; *5d*, 9:21, very strong current, dull sensation; *5c*, 9:23, strong current, dull sensation; *5f* and *g*, 9:25–:27, strong current, same dull sensation.

all the time in the pyloric antrum of the stomach—the area of colicky pain continued to hover around the umbilicus. Similarly, when the electrode was drawn up from the pyloric antrum into the cardiac region of the stomach (Experiments 6 to 8, Figure 7), pain was still referred to the umbilicus. This was the more surprising since we were dealing with that case of ptotic stomach, in which, previously, the site of pain had descended with the electrode (Figure 2, Experiments 9 and 10). However, after the subject had arisen and walked from the x-ray table to the fluoroscopic room, and some twenty minutes had elapsed, apparently a new judgment was established, for a new site of pain was localized—namely the one approximately over the electrode (Experiment 9, Figure 8).

These observations seemed to point to a conditioning of nervous pathways—to a temporary selection of one out of many avenues; and, in so doing, it destroyed the simple expectancy that pain arising from a given portion of the gut could be projected onto the abdominal wall with any degree of accuracy.

By the process of elimination these conclusions also focussed attention on the possibility that pain in the gut was being projected to the skin of the abdominal wall from the viscera, notwithstanding its apparent deep location. In this, we were directed by the very significant experiments of Weiss and Davis (5). These authors found that in patients suffering from deep yet definitely localized spontaneous pain (accompanying such disorders as gastric ulcer, acute appendicitis, chronic cholecystitis, etc.), intradermal injection of 2 per cent novocain abolished the pain for several hours. Accordingly, we undertook to block out the areas of skin overlying the site of the pain that accompanied electrical excitation of the gut.

5. *Effect of anesthetizing the skin.* The first attempts are indicated in Figures 5 and 6. On two different days (Experiment 5) pain was observed to persist under areas of the skin that had been infiltrated with novocain. However, as it was subsequently ascertained by pharmacological tests that this particular subject was four times as resistant to novocain as the average medical student, these experiments were not deemed conclusive. Accordingly, they were repeated in another student, the one previously discussed in connection with Figure 7.

This time, with the electrode just above the angular incisure of the stomach, a colicky pain was localized in the left epigastrium (dot, Experiment 9, Figure 8). Then Area I (Figure 8) was injected both intradermally and subcutaneously with 1 per cent novocain (and adrenalin).[5] When stimulation was resumed, with the electrode in the same place (Experiment 10), the same degree of pain was felt as before, but this time it

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[5] The authors are greatly indebted to Dr. Owen Wangensteen, Chief of the Surgical Service in the University Hospital, for his skilful administration of novocain.

Electrode in Stomach
Case D.S.F., Series III

A.
Before
Novocain

Subject prone except Exp 4 e 5

B.
After
Novocain
(except Exp 9e11)

Subject supine

Fig. 7

Fig. 8
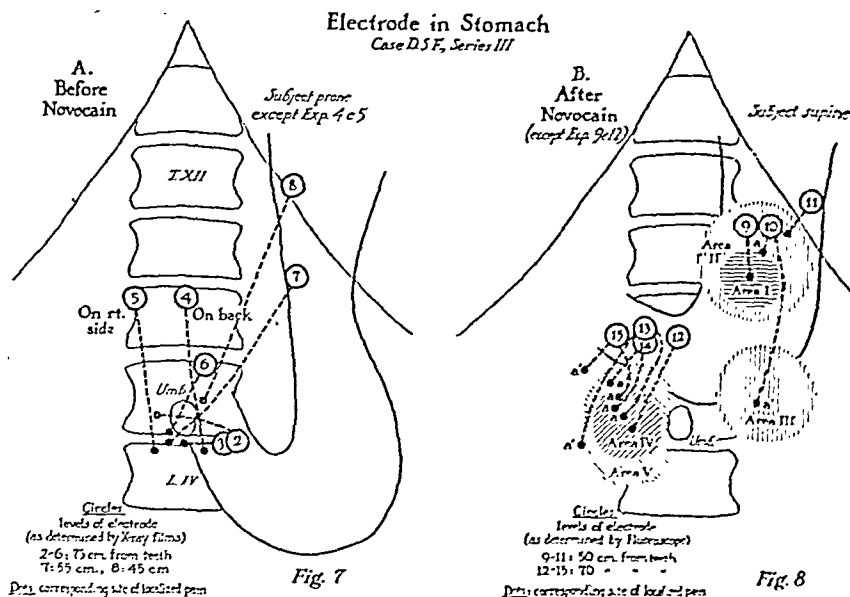
FIG. 7. GROUP OF EXPERIMENTS ILLUSTRATING FAILURE OF PAIN AREA TO MOVE WITH CHANGE IN POSITION OF ELECTRODE. CASE D. S. F., SERIES III (APRIL 16, 1934)

Arabic numerals indicate sequence of experiments: *1* (same position as *2*), *7*:16 a.m., weak current (2d electrode on left calf), no visceral sensation, yet abdominal rigidity; *2, 7*:21, moderate current (as before) barely felt; *3, 7*:23, moderate current (2d electrode on right forearm); fluttering sensation stronger than before; *4, 7*:30, moderate current (2d electrode on right forearm), colicky pain; *5, 7*:33, moderate current (2d electrode on right calf), "knocking" sensation increasing to colic; *6, 7*:49, moderate current (2d electrode on right calf), vibrating sensation painful at end; *7, 7*:53, moderate current (2d electrode on left calf), same sensation as at *6*; *8,* 8:05, moderate current (2d electrode on right calf), unpleasant sensation suggesting nausea but not painful; *9* (Figure 8), 8:25, moderate current, colicky pain.

FIG. 8. SERIES III CONTINUED (APRIL 16, 1934): ILLUSTRATING THE MIGRATION OF PAIN FROM ANALGESIC AREAS OF THE SKIN

9, 8:25 a.m., moderate current, colicky pain; 8:30, Area I rendered analgesic by intradermal and subcutaneous injection of 1 per cent novocain: *10,* 8:35, moderate current, same sensation as at *9*, but site of pain moved during period of stimulation (10 seconds) from *10a* to *a'*; 8:37, Areas II and III anesthetized; *11,* moderate current, colicky pain felt under Area II; *12,* 8:52, moderate current, colicky pain and throbbing sensation, increasing in intensity; 8:53, Area IV rendered analgesic; *13,* 8:58, moderate current, sensation stronger than before and quite painful, felt simultaneously at *a* and *a'*; *14,* 8:59, ditto; *9:60,* Area V anesthetized; *15,* 9:10, moderate current, same sensation as before: this time felt first at *15a*, then moved to *a'*, though still remaining at *15a*.

The second point, as to why colicky pain that originates in the stomach and duodenum is referred to the extreme anterior terminations of certain intercostal nerves and not to the lateral rami or to the posterior divisions of these nerves (Figure 9), suggests that there is a fundamental anatomical arrangement whereby visceral afferent fibers, such as neurones *a* and *b,* arborize in that part of the spinal cord or spinal ganglion where neurones of the anterior rami are located.

The interesting experiments of Bloomfield and Polland (10), to which our own may be said to be complementary, also tend to confirm this view.

Comparison of Effects of Distension *(stippled areas)*
with Faradic Excitation *(dots)*

A. Balloon or electrode in upper duodenum at level ⊗

B. Balloon or electrode in lower duodenum at level ⊗

• 1 case at inguinal ring (on back)

▫ 9 cases at inguinal ring (or scrotum) (5 on abd.; 4 on back)
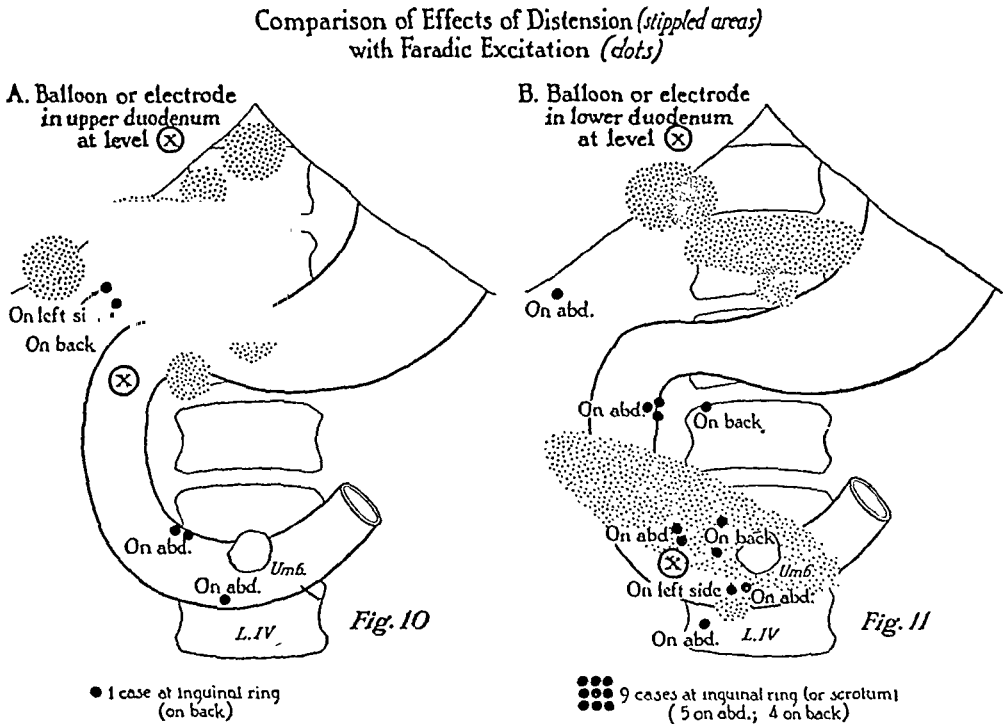
Fig. 10.   Diagrams Illustrating Effect of Tension Exerted upon Intestinal Muscle in the Region of the Right Upper Flexure of the Duodenum (*x*)

Stippled areas, site of pain following distention of duodenum by the balloon method, with patient erect (Bloomfield and Polland, 1931); *dots,* site of pain following faradic excitation of same portion of duodenum.

Fig. 11.   Diagrams Illustrating Effect of Tension Exerted upon Intestinal Muscle in the Region of the Right Lower Flexure of the Duodenum (*x*)

Stippled areas and *dots* indicate respective sites of pain resulting from distention and contraction of this portion of the duodenum.

For when balloons were lowered into the stomach and duodenum and inflated with air, the patients described the pain as being deep-seated, yet as always lying under the anterior wall between the xiphoid process and the umbilicus.

In the experiments with the stomach, their results differed from ours chiefly in the fact that the site of distress was not sharply localized, the patient referring to the area by placing his whole hand over the mid-epigastrium instead of pointing to an area with his finger. Also the sensations were less definable and were related to symptoms arising from overloading the stomach rather than to colic. Presumably the larger area of referred pain was directly related to the fact that the area of stomach wall subjected to pressure by the balloon (200 to 500 cc. air) was much greater than that subjected to faradic excitation. Distention of the duodenum, on the other hand, resulted in much more definitely localized pain (Figures 10 and 11) than in the experiments with the stomach. We may infer that this was due to the smaller size of the balloon (40 to 200 cc. air). Even so, the areas pointed to after distention were somewhat larger than those pointed to after faradic stimulation. Yet in neither case did maximum muscle tension cause the pain to be referred to the sides or back of the trunk. The latter phenomenon, when observed clinically, must therefore be due to extension of the lesion into the mesenteries or retroperitoneal tissues.

The *third point*, as to why localization of such pain is variable—being felt sometimes at one, sometimes at another portion of the anterior abdominal wall—may be explained, in part, by Sherrington's demonstration of the overlapping of sensory fields in the trunk (see Ranson (9), p. 59).
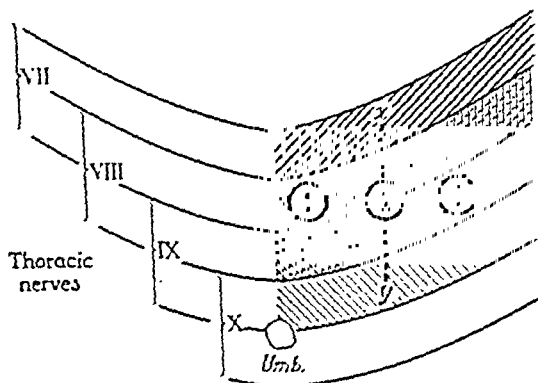
FIG. 12. DIAGRAM (AFTER RANSON, FROM SHERRINGTON) ILLUSTRATING THE OVERLAPPING OF SENSORY FIELDS OF THE ABDOMINAL WALL.

Vertical lines, zone of thoracic nerve VIII; upper oblique lines, zone of nerve VII; lower oblique lines, zone of nerve IX; *Umb.*, umbilicus. Area 1 supplied by nerves VII and VIII; 2, by VII, VIII, IX; 3, by VIII and IX. (For explanation of *x* and *y*, see below.)

As shown in Figure 12, the zones of the intercostal nerves dovetail in such a way that Area 1, for instance, is supplied by afferent neurones from both the seventh and eighth, Area 2 from the seventh, eighth and ninth,

and Area 3 from the eighth and ninth thoracic nerves. As related to faradic stimulation of the stomach, this means that when a subject has localized pain at Area 2 the impulses from the gut may have entered the cord from splanchnic nerves VII, VIII, or IX, or from all three. If the impulse through VII predominated, the pain might have been referred to Area x instead of Area 2; or if that through IX predominated it might have been referred to Area y instead of Area 2; but if all three were approximately equal, pain from the stomach might have been localized anywhere from costal margin to umbilicus.

Variability similar to that which we encountered, has also been recorded by Bloomfield and Polland (10), in experimental distention of the duodenum (see Figure 11). Rivers (11), also, seems to have been faced with the same problem, for in certain gastric and duodenal ulcers he found that pain was usually located to the left or right of the umbilicus, whereas in others it shifted to the left or right costal borders, respectively. He would interpret this on the basis of the depth of the lesion, the implication being that different nerve endings of the gut are involved in these two types of cases. Occasionally, also we have noted that increasing the duration of the stimulus (Experiments 4b vs. 4c, Figure 4) or increasing its strength (5a vs. 5e, Figure 6) has changed the site of pain; but in these cases the presumptively deeper penetration of the current caused the pain to appear at the umbilicus, and the lesser penetration at the costal border—just the reverse of Rivers' findings. Then there is the peculiar situation shown in Figure 7 where the site of pain remained at the umbilicus regardless of the changing nature of the pain, the shifting of the body, or even the shifting of the electrode.

Apparently, therefore, there is some other factor of selection that must be reckoned with. Thus Polland and Bloomfield (12) in their experiments with distention of the esophagus have shown that there are sites of predilection which are not related to the position of the balloon; for out of 191 times in which the tube was inflated just enough to produce a minimal stimulus, pain was localized 87 times at the lower end of the sternum and 48 times just above the suprasternal notch—regardless of the part of the esophagus in which the balloon was situated. Further inflation caused the pain to spread widely or to appear in a new site. Also they found, as we did, that sometimes a constant stimulus in the same individual gave different results.

Similarly, Weiss and Davis (5) have described a typical case in which distention of the lower third of the esophagus caused severe pain between the shoulder blades at the level of the 6th thoracic vertebra. After infiltrating this area of skin with novocain, pain appeared over the 7th thoracic vertebra; when the latter area was infiltrated, pain appeared over the 4th thoracic vertebra. Finally when this area was injected with novocain (all

the previous areas being analgesic) slight pain was still felt in the back, but severe pain was felt, anteriorly, over the sternum.

What is the nature of this order of selection? Is it primarily mechanical, depending upon the juxtaposition of nerve endings in the gray matter of the nervous system; or quantitative, depending upon the number of nerve endings from a given nerve at the point of stimulation; or physiological, depending upon such factors as threshold and Bahnung? Or is it a combination of one or more of these factors? The impression that we have gained from these experiments is that it is more than all these and that it involves an integrative process going on in the higher centers.

This brings us to *the fourth and fifth points* raised by our experiments —namely as to why projection of pain on the abdominal wall tends to follow the course of the gut and yet why, at certain times, it stays in one area regardless of shift of posture or regardless of which segment of the gut is being stimulated.

Unless one accepts the parietocutaneous theory of Morley (vide infra), the interpretation that would seem to fit all the facts most closely is that localization of pain arising from tension of the visceral musculature is the result of integration—a " putting together " by the higher centers of two sources of information, one coming from visceral neurones *a* (Figure 9) and the other from somatic neurones *c*. This implies a training from birth in the association of impulses carrying true visceral pain with those that are projected from adjacent areas of the skin. Also, if integration be admitted, then we can explain, on grounds of conditioning, such otherwise inexplicable phenomena as have been recorded in Figure 7.

Regarding the latter case it might be said that when localization occurred in the umbilical region, it represented true visceral (protopathic) pain; but when it was localized in the left epigastric region it was due to a spread of the current to the peritoneum of the anterior abdominal wall,— from which point it was referred to the overlying skin by a parietocutaneous radiation (see Morley's interpretation of the two kinds of pain in appendicitis).

However, in addition to the reasons already given for believing that the current does not spread from the inside of the gut to the anterior peritoneum (p. 838) there are experimental grounds for questioning Morley's hypothesis. For instance, when he repeated the work of Weiss and Davis he found that while spontaneous pain and hyperalgesia were abolished by intradermal injections of novocain, deep tenderness, pain on coughing and muscle rigidity remained (Morley (13)). Obviously, deep tenderness in these cases could not have been due to a parietocutaneous radiation, otherwise it would have been modified by cutaneous anesthesia. Especially noteworthy was Case 4, of Morley's series, in which the appendix was retrocecal in position and therefore not in contact with the peritoneum of the anterior wall. So also with his ulcer experiments: anatomically, it is

impossible, by pressing upon the abdominal wall, to bring that portion of it that lies immediately over the pylorus into contact with the duodenal cap. The liver intervenes. Therefore, if the pain of deep tenderness in such cases was not felt directly in parietal nerve endings, it must have arisen in the mesenteries (Sheehan (14)), or in retroperitoneal tissues of embryologically adherent mesenteries, and so have passed into the cord via the splanchnic nerves (e.g., neurone *d*, Figure 9).

Furthermore, it has never been proven experimentally that nerves of the anterior parietal peritoneum do not register pain directly, i.e., without the intervention of Morley's parietocutaneous radiations. On the contrary, Capps and Coleman (4) have reported that when the anterior peritoneum is pricked by wires inserted through trochars embedded in the abdominal wall, the sharp stitch-like pains are localized within half an inch of the end of the wire. (Incidentally, this area of skin should be injected with novocain to ascertain whether the skin is involved at all in this type of pain.) Also there is some evidence that different portions of the peritoneum behave differently. Thus Capps and Coleman have noted that when they touched the periphery of the diaphragm with a wire, the pain was quite different than before; for it became diffuse and was indicated by the patient's placing his hand over the hypochondrium.

The sixth and seventh points raised by our experiments—namely as to why the site of pain migrates from an area of skin that has been anesthetized and simultaneously persists under this area—are best explained by reference to Figure 9. One must assume that when the cutaneous endings of neurones *c* are anesthetized, the conductivity or thresholds of these neurones are sufficiently changed (be it ever so slightly) as to eliminate them from competition and to give precedence to adjacent neurones that are simultaneously being bombarded by splanchnic impulses. For example, suppose that Area 2 (Figure 12), which is supplied by peripheral nerves VII, VIII and IX, is anesthetized. Then the pain arising from bombardment of nerves VII, VIII and IX could appear at *x* or *y* or any other portion of the skin supplied by the anterior rami of these nerves.

The persistence of pain under the analgesic area may be explained in four ways: 1—by the bombardment in the ganglia of peripheral neurones coming from deeper layers of the abdominal wall than neurones *c* (this is not considered probable in view of the experiments of Weiss and Davis); 2—by the continuance of impulses from the cell bodies of neurones *c*, it being assumed that anesthetizing their cutaneous endings may have altered but not abolished the conductivity of these neurones; 3—by the continuance of impulses from neurones *a* which had previously conditioned the higher centers to associate the sensation of visceral pain with the area now anesthetized; or 4—by impulses from neurones *a* which are directly felt in the hollow viscera.

That true visceral pain may persist in the absence of peripheral im-

pulses is clear from the experiments of Davis, Pollock and Stone (15). These investigators found that after section of all thoracic nerves in cats, the animals still gave evidence of pain when the gallbladder was distended experimentally. It seemed to them, however, that the nature of the pain was somewhat modified. This was not true in our experiments. Therefore one would like to know whether, under such conditions, the localization of pain was modified. Perhaps such needed information may be obtained through the cooperation of patients whose intercostal nerves have been sectioned by thoracoplasty.

Finally, the experiments of Weiss and Davis (5) suggest that pain arising from inflammatory lesions of the gut may have a different mechanism from that arising from excessive muscle tension of the gut. Thus they found that in such inflammatory conditions as acute appendicitis, cholecystitis, etc., spontaneous pain and hyperalgesia were abolished by intradermal injections of novocain. This suggests that what they accomplished by local anesthesia was interference with viscerocutaneous reflexes and not with viscerocutaneous radiations (as in our experiments).

Returning to Figure 9 one may postulate that a visceral afferent impulse arising in one of the tunics of a diseased organ, perhaps in the arteries of that organ (Moore and Singleton (16)), would pass to the lateral column of the cord (neurone x), then be shunted out to a sympathetic ganglion (neurone y) and then be relayed to the skin (neurone z) where it would set up disturbances in the endings of parietal nerves (neurone c'). Thus pain from an inflamed organ might produce superficial tenderness and appear to come from the skin, yet be abolished by anesthetizing the cutaneous endings of postganglionic fibers (neurone z).

This is the theory of Verger (17), whereby an algogenic stimulus arising in the viscera produces a vasomotor reflex that modifies the " vascular bouquet " of the skin, thereby exciting sensory endings in the skin that are supplied by cerebrospinal nerves. A somewhat comparable theory has been proposed by Sfameni and Lunedei (18). This postulates that algogenic impulses arising in the viscera stimulate efferent neurones in the cord that terminate within sensory corpuscles of the skin,—the so-called " apparatus of Timofeew " (Maximow (19)), thereby setting up physicochemical changes in the cerebrospinal components of the corpuscle. Such theories as these remove the *a priori* objections of Morley to viscerocutaneous reflexes and radiations and would seem to open the door to a more neurological approach to the problem of visceral pain.

SUMMARY

1. An experimental method has been devised whereby the gut may be stimulated, electrically, through the metal end of a stomach tube.

2. As observed under the fluoroscope, excitation with a tetanizing current usually causes ring contraction of the stomach and duodenum.

3. Such spastic contraction is accompanied by sensations ranging from barely perceptible feelings of pressure to severe colicky pain.

4. Such sensations are definitely localized under the upper quadrants of the abdomen—the subject pointing to the spot with his finger.

5. As the electrode is drawn up through successive portions of the duodenum and stomach, the sites of pain progressively outline the position of these organs; but there is considerable aberrancy (Figures 1 and 2).

6. When the electrode is kept in one segment of the gut, but the body posture is changed, the site of pain usually shifts with it (Figure 3). Sometimes, however, the pain remains localized in one region after both the electrode and the body posture have been changed (Figure 7). This is interpreted as a temporary conditioning of the nervous pathways.

7. When an area of the skin to which the patient has pointed is anesthetized, the pain migrates to a position outside the area, thus revealing that cutaneous nerves are involved in spastic contraction of the gut.

8. These experiments are interpreted to mean that localization of visceral pain arising from spastic contraction of the gut is a viscerocutaneous radiation due to splanchnic bombardment of somatic neurones.

9. At the same time that the pain migrates from an analgesic area it continues to be felt under that area. The similar persistence of visceral pain in animals after section of all thoracic nerves (Davis, Pollock and Stone) suggests that perhaps visceral pain is normally an integration of impulses from both splanchnic and cutaneous sources and explains why such pain tends to follow the course of the gut. Confirmation of this theory awaits experiments with patients in whom the thoracic nerves have been cut.

10. The experiments of Weiss and Davis in abolishing pain from diseased viscera by anesthetizing a localized cutaneous area suggest that the mechanism of pain arising from inflammation may be different from that caused by spastic contraction or distention of the gut. Their work points to an excitation of the skin by reflexes (originating in the viscera) which in turn set up centripetal impulses in the cutaneous endings of peripheral nerves. On the other hand, Morley's inability to abolish deep tenderness by cutaneous anesthesia suggests that such pain is not a parietocutaneous radiation but parietal pain localized in situ or else pain arising in mesenteries or gut which is being transmitted to the cord by splanchnic nerves.

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UNEXPLAINED FEVER IN HEART FAILURE

By ALFRED E. COHN and J. MURRAY STEELE

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HISTORICAL INTRODUCTION

Very early the occurrence of fever during disease was commented upon by Hippocrates as one of its most important symptoms but not until more than 2000 years later, in the early part of the seventeenth century, were quantitative measurements of the temperature of the body obtained. Sanctorius employed a thermometer of his own making for this purpose. Still another century elapsed, however, before the significance of such measurements began to be recognized. Boerhaave occasionally used a thermometer as a practical aid in diagnosis but left for his pupils Van Swieten and De Haen, the first professor of clinical medicine at Vienna, the chance to record the useful, if naive, observation that external temperature was more easily recognized by the thermometer but that internal temperature was still best described by symptom and sign. It is of considerable interest that "internal heat" or temperature was described by the iatro-physicians in terms of disturbance of circulation and its presence was decided upon by the frequency and quality of the pulse. One view accounted for the production of heat mechanically by the friction of blood moving in the vessels but was opposed both by De Haen and John Hunter. When Lavoisier and Laplace announced (1780) that the source of animal heat was combustion of organic substances in the body, the theory of mechanical production of heat was quickly discarded in favor of one chemical in nature. The belief of Lavoisier that heat was generated in the lungs during oxygenation of the blood was resisted by Brodie (1811) who looked for its source in the nervous system. There followed many observations on the relation of the nervous system to the temperature of the body by Chossat (1820), Edwards (1824), Home (1825), and later Claude Bernard (1852). These researches succeeded, not in establishing the location of the source of heat, but in leading to an understanding of the extraordinary degree of control which the nervous system exerts over the temperature of the body.

While these physiological studies of the regulation of temperature were being carried out, records of the temperature of the body were being obtained by practicing physicians. In France, Piorry, Gierse, Roger; in England, Martine, Currie and Davy; in Germany, Zimmermann, Traube and von Bärensprung made numerous observations in health and in disease. Attempts to obtain more than one or two records of temperature during the course of an illness were rare although it is true that Davy, in mapping out physiological variations, and Traube, in attempting to follow closely the influence of taking digitalis on the temperature of the body and the course of events on critical days in febrile illnesses, occasionally made frequent measurements during the twenty-four hours.

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Wunderlich (1) was the first, however, to recognize clearly and to demonstrate the diagnostic and prognostic importance of a series of measurements taken throughout the course of a disease. He recorded the temperature of patients usually as often as every four hours and constructed charts showing its variations. He published clear descriptions regarding changes in the temperature of the body during many febrile diseases. The temperature charts of today are an expression of the universal recognition of the value of this procedure and of the technical convenience afforded by the use of Sir Clifford Allbutt's recording clinical thermometer.

Wunderlich's method of taking measurements frequently enough to obtain curves of the course of the rectal temperature characteristic of different diseases was imitated rapidly in England by Ogle, Aitken, and Gibson. Jurgenson and Wunderlich's pupils established it in Germany. The period in the nineteenth century that witnessed the introduction of thermometry into clinical medicine was characterized also by significant advances in knowledge concerning infectious diseases. The form of the record of temperature of the body became one of the principal means of characterizing various febrile affections and of recognizing complications of these diseases. The close association of infection with fever was soon established and stressed so energetically that at present physicians find it difficult to dissociate the occurrence of fever from its usual association with an infectious ailment.

It is well recognized, however, that many conditions other than infectious disease may be associated with elevation of the body's temperature, such as injury to certain parts of the brain, extravasation of blood into tissue spaces, infarcts, the state of hyperthyroidism and of sunstroke in which there is no reason to suspect the presence of infection.

When fever is observed in patients suffering from heart failure its nature has usually been ascribed to some sort of cryptic infection. And since patients with heart failure frequently suffer simultaneously from infection, the two conditions do in fact often coexist. Sometimes neither infection nor a non-infectious condition recognized as commonly associated with fever can be demonstrated. Under these circumstances physicians have generally assumed that infection is present but that the methods available are insufficient to disclose the nature or the location of the process. Because of the similarity of the physical signs of pulmonary infections and chronic passive congestion of the lungs, the lungs are usually regarded as infected though the possibility is recognized that infectious foci may exist in other organs as in the tonsils, the kidneys, or indeed, according to Romberg (2), in the heart itself.

Observations of the behavior of certain cardiac patients with fever have brought into question the wisdom of assuming in instances of unexplained fever the presence of an infectious process. For this reason the records of 368 cardiac patients coming under observation between 1914 and 1929 have been studied. Of 172 who presented symptoms or signs of heart failure, 153 exhibited, on two or more occasions, elevation of the rectal temperature to at least 100° F. Usually the elevations were clearly associated with con-

ditions generally recognized as accompanied by fever, but in 49 cases the occurrence of fever was without satisfactory explanation. In certain ones its development suggested an origin, at least in part, dependent upon heart failure itself.

Five cases are described to illustrate this conception:

Case I. G. M., Hospital Number 2098, male, aged 50 years, was admitted to hospital May 19, 1914, complaining of shortness of breath, weakness, and pain in the limbs.

His family history did not contribute to an understanding of his illness. His health had been excellent. Measles and pertussis in childhood, gonorrheal urethritis at the age of twenty and pain in the joints without swelling for five weeks at forty-three years of age had been his only illnesses. Two weeks after an attack of grippe 2½ months before admission, edema of the legs, pain in the back, shoulders and legs, dyspnea and weakness made their appearance. In retrospect he believed that he had been slightly short of breath for a year. He was an elderly, well-developed man, restless and slightly dyspneic. A muco-purulent post-nasal discharge was observed. The heart was enormously enlarged, the maximum transverse diameter of its shadow measuring 20.5 cm. Auricular fibrillation was present; a systolic thrill was felt and systolic and diastolic murmurs were heard at the apex. The systolic blood pressure measured 189 mm. Hg and the diastolic 100. The lungs were clear. The liver extended only 1 cm. below the costal margin. There was no edema of the legs and no cyanosis. The urine contained albumin and the sediment, a few red and white blood cells and numerous hyaline and finely granular casts. The Wassermann reaction of the blood was negative.

During the first two weeks he grew slowly worse; edema increased, the liver enlarged, rales were heard at the bases of the lungs, and the cardiac rate became accelerated. On June 3, digipuratum was given, followed by diuresis and disappearance of these signs. The administration of digipuratum was then omitted. During the next ten days the symptoms and signs gradually reappeared together with elevation of the rectal temperature. All symptoms were again relieved by the administration of the tincture of digitalis, begun July 1 (Fig. 1). The fever also subsided. During the presence of fever no obvious source of infection was found. The upper respiratory passages were normal and no signs of consolidation appeared in the lungs. The urine was clear. He was discharged July 17, 1914, fairly well and was instructed to continue to take the tincture of digitalis. He was readmitted on September 2, 1914. Periods during which digipuratum was given alternated with others free of it. When he was without digitalis edema and dyspnea increased, rales were heard in the lungs, the liver enlarged, the frequency of the heart beat increased, cyanosis made its appearance and the temperature of the body became elevated. Administration of the drug was followed by the disappearance of these signs and symptoms. Two of these attacks of failure, the first culminating on the 16th October, the second on the 19th November, 1914, were accompanied by the appearance of jaundice. The day after the first of these peaks dullness at the base of the right lung posteriorly and alteration of breath sounds were found. These signs persisted until November 28, and were apparently due to accumulations of fluid. On December 12, 1914, he was discharged free of the signs of heart failure. He was admitted again on May 24, 1915, in a state of extreme heart failure with Cheyne-Stokes breathing, jaundice and fever (Fig. 1,
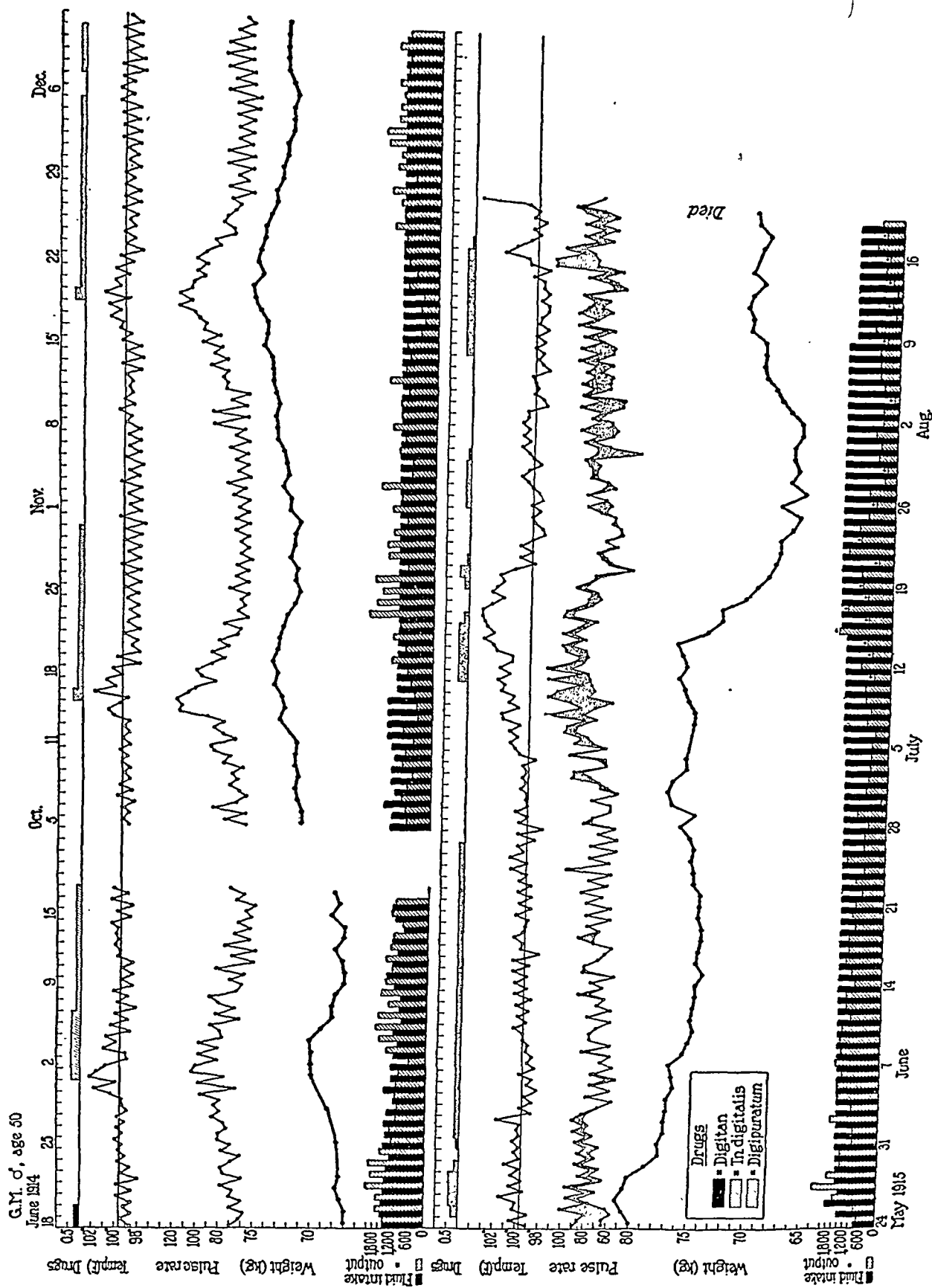
FIG. 1. PORTIONS ARE SHOWN OF THE HOSPITAL RECORDS OF CASE I

The stippled areas in this and all of the following charts indicate the pulse deficit at the radial artery.

lower half). Taking digitalis was followed by some improvement and disappearance of fever. The ground gained was rapidly lost when its administration was stopped because of the development of coupled rhythm. Renewed development of edema, increase in pulse rate, dyspnea and cyanosis and elevation of the temperature of the body recurred so promptly that diuretin was administered. Some decrease of the edema took place. As soon as the coupled rhythm disappeared, digipuratum was given again. The improvement which occurred was short lived however and on August 21, the patient died. Postmortem his heart was enlarged. There was chronic passive congestion of the liver, kidneys and lungs; right hydrothorax (200 cc.), ascites (300 cc.), and old fibrous pleural adhesions on both sides were found.

Case II. M. C., Hospital Number 4425, male, aged 33 years, a shipping clerk, was first admitted on December 1, 1921, complaining of heart trouble. The family history was of no significance. The past was free of any ailments including those of the "rheumatic group." He fell ill in June 1918, at age 29, with cough, shortness of breath and occasional hemoptyses. A physician told him that he had heart trouble. He stopped work for six months, two weeks of which were spent in the Beth Israel Hospital. Later five similar attacks occurred during the last of which he was admitted to the Hospital of the Rockefeller Institute, two months after the onset of symptoms.

All the abnormal physical signs were referable to the cardiovascular system. A rapid precordial "undulation" was noted. No shocks or thrills were felt. The widest measurement of relative cardiac dullness extended 4.5 cm. in the 4th interspace to the right and 11.5 cm. in the 5th interspace to the left of the midline. The rhythm was rapid and altogether irregular (auricular fibrillation). The pulmonic second sound was louder than the aortic. No murmurs were heard. The systolic blood pressure measured 108 mm. Hg and the diastolic 86. There was no enlargement of the heart nor was there an unusual contour in the x-ray photograph. The radial pulse was of fair volume; the rate was 88 per minute while that of the apex was 132. The edge of the liver was felt about 6.0 cm. below the costal margin but was not tender. There was no edema of the extremities. The urine contained considerable albumin but no casts or red blood cells. Benedict's solution was faintly reduced in one of many examinations. The Wassermann reaction of the blood was negative.

After 3 days during which the apical rate varied between 130 and 190, with a pulse deficit of 50 to 100 per minute, and the rectal temperature ranged from 100 to 101.8° F., dyspnea and cyanosis became so intense that digitan (Merck) 2 grams, was administered in the next succeeding 48 hours. The rapid cardiac rate, fever, and dyspnea subsided, cyanosis disappeared and in spite of the fact that no visible edema was present diuresis occurred with a loss of 9 kilograms in 8 days. Ten days later the administration of quinidine sulphate 1.2 gram was followed by return of the normal rhythm. On January 25, 1922, free of any signs of heart failure he was discharged. He remained well without medication until June 1925, when auricular fibrillation recurred. He was given a large amount of digitalis to take, before being readmitted to observe the effect of administering quinidine. On June 22, 1925, 1.2 gram of quinidine was given, and on June 28, 1.6 gram without success in restoring normal rhythm. After taking 2.0 grams on June 29, reversion to normal rhythm took place during the night (Fig. 2). Since then and until June, 1933, the rhythm of the heart has been normal.
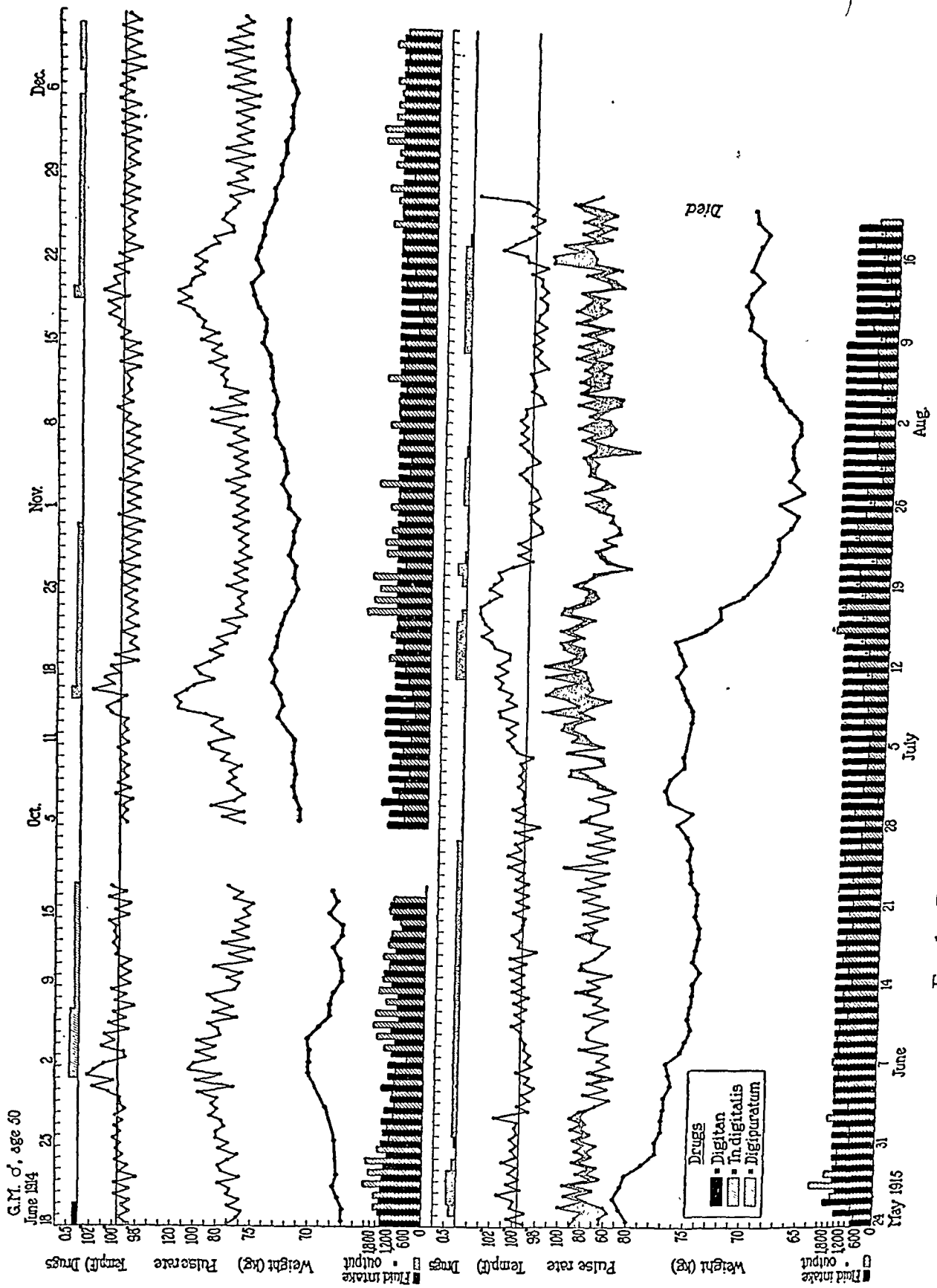
FIG. 1. PORTIONS ARE SHOWN OF THE HOSPITAL RECORDS OF CASE I

The stippled areas in this and all of the following charts indicate the pulse deficit at the radial artery.
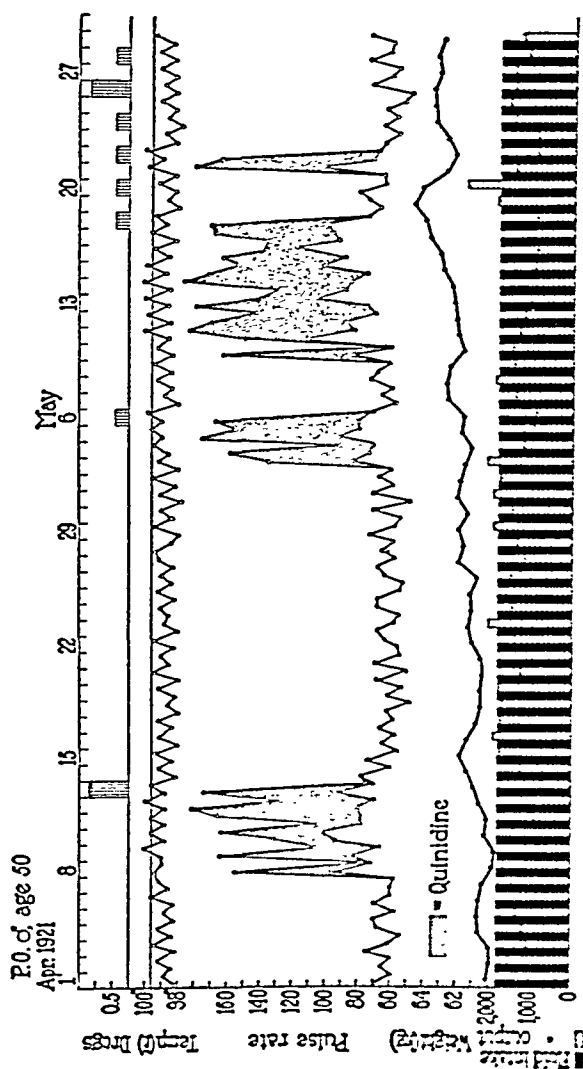
FIG. 3. THE CHART OF CASE III IS REPRODUCED IN WHICH ARE DISPLAYED SEVERAL ATTACKS OF PAROXYSMAL AURICULAR FIBRILLATION

Case III. P. O., Hospital Number 3968, aged 53, male, was admitted o: September 29, 1919, complaining since May, 1919, of palpitation and a sense of oppression in the chest. He had a chancre at the age of 23 treated with medicine by mouth for a year, and ten years later another penile lesion treated
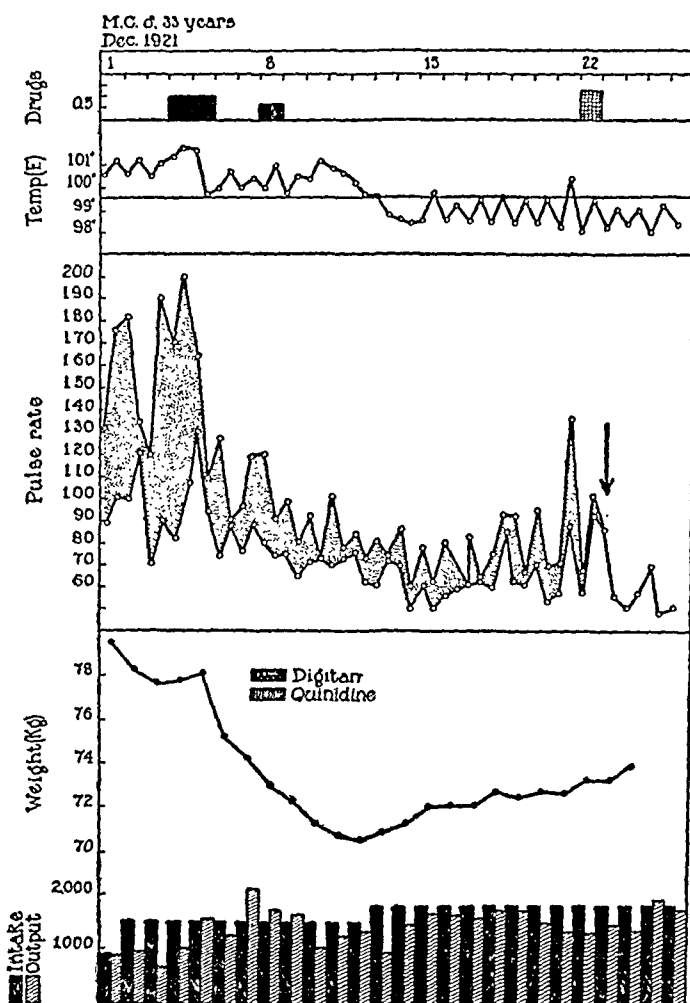
FIG. 2. THE FIRST PORTION OF THE RECORD OF CASE II IS EXHIBITED

An elevation of temperature on December 21 accompanies increase of the cardiac rate after the injection of atropine sulphate intravenously.

by local fulguration. At the age of 46 years an attack of rheumatism (?) occurred with painful swollen joints but no fever. At age 52 a similar attack involved only the right knee. The palpitation and the smothering sensation in the chest which appeared May, 1919, were quickly followed by dyspnea on exertion and edema of the feet which resulted in his stopping work.

His general physique and nutrition were good. No dyspnea, orthopnea, cyanosis or edema was noted. Advanced dental caries and pyorrhea were present. The heart was enlarged, the sounds clear. There were no murmurs. The totally irregular rhythm was due to auricular fibrillation. The rate at the apex was 105, the pulse deficit 37. The systolic blood pressure measured 142 mm. Hg and the diastolic 70. The peripheral vessels were somewhat thickened. The lungs were clear on percussion but a few moist râles were heard at both
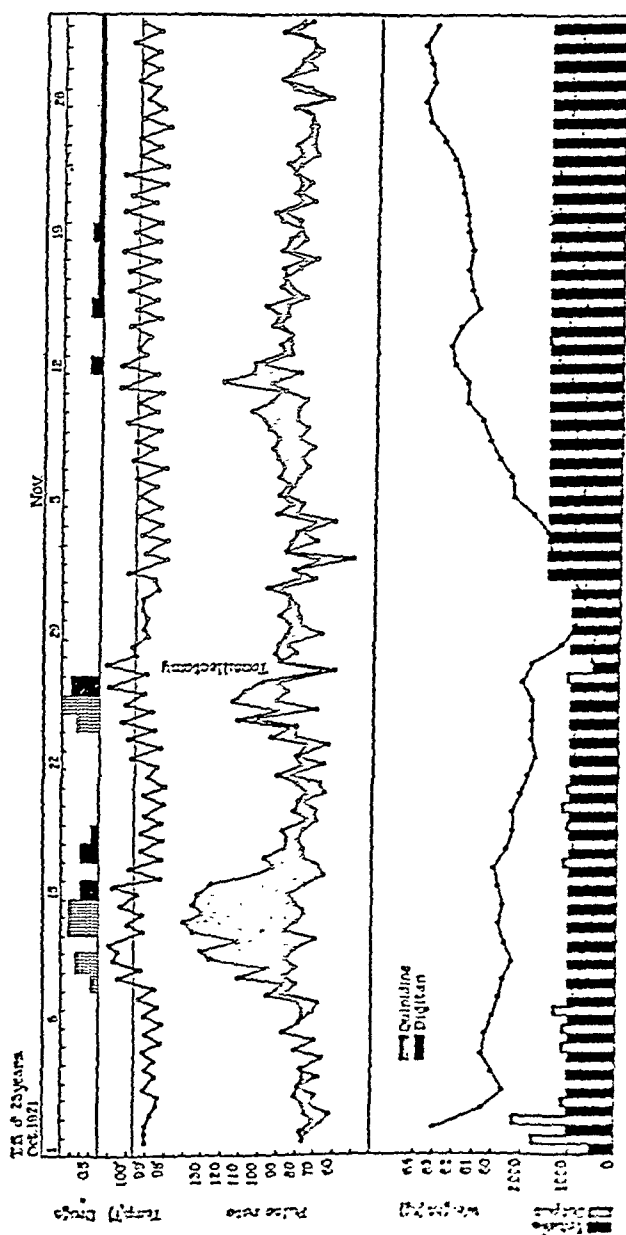
FIG. 4.   IN THE CHART OF CASE IV, FEVER AND TACHYCARDIA FOLLOW THE ADMINISTRATION OF QUINIDINE ON TWO OCCASIONS

bases. The edge of the liver was felt 1 cm. below the costal margin. The urine contained a faint trace of albumin. The Wassermann reaction of the blood was positive on several occasions.

For a year and a half the patient was observed in and out of the hospital. The auricles continued to fibrillate but by the judicious use of digitalis he was kept free of the symptoms of heart failure. He received also mercurial inunctions. His course was practically afebrile save during his first attack of mild failure. In March, 1921, he was readmitted to receive treatment with quinidine. Signs of heart failure were not present. Palpitation on exertion was the only complaint. On March 15, after the administration of 4.6 grams of quinidine, the heart rhythm reverted to the normal mechanism. The normal mechanism was, however, interrupted four times by paroxysms of rapid auricular fibrillation (Fig. 3). With each there occurred dyspnea, palpitation, gain in weight and fever. With their disappearance the weight promptly fell, palpitation, dyspnea and fever subsided.

Subsequently he took quinidine daily and the rhythm continued regular. He remained in excellent health until January 9, 1923, ten days after he had stopped taking quinidine on his own initiative. On this day auricular fibrillation recurred. He was taken to another hospital and died in May, 1923.

Case IV. T. S., Hospital Number 4390, male, aged 23 years, an elevator operator, was first admitted to hospital on October 1, 1921. His chief complaints were shortness of breath and sweating. The family history contributed no information of importance. His only illnesses were a severe attack of " inflammatory rheumatism " at age 9, with markedly swollen, red joints, and influenza at age 22. The latter illness was followed by weakness which prevented his returning to work for two months. In 1917, and again in 1919, he was rejected for service in the Army and the Navy because of heart trouble. Shortly thereafter he became short of breath, but continued working until April 1921 (6 months prior to admission), when he suffered an attack of acute appendicitis for which appendicectomy was performed. He was again told that he had heart disease and was given digitalis. From this time on he was in bed most of the time because of severe dynspnea, orthopnea, and cough and, for four or five weeks prior to admission, edema of the legs.

His heart was enlarged, its rhythm that of auricular fibrillation. A blowing systolic and rumbling diastolic murmur were heard near the apex and the second pulmonic sound was markedly accentuated. The radial pulse was of poor and varying volume. The rate at the apex was 78; at the radial artery 70. The blood pressure measured 100 mm. Hg systolic and 60 diastolic. A few crepitant râles in the right axilla and occasional sibilant râles were heard. The edge of the liver was felt 1 cm. below the costal margin. Edema was found only over the sacrum. The urine on examination was found to be normal. The Wassermann reaction of the blood was negative.

After four days in bed edema disappeared, the lungs cleared, and the degree of dyspnea decreased. Quinidine sulphate was administered; 0.4 gram on October 10, 1.2 gram on October 11, and 1.6 gram each on October 13 and 14. The heart rate increased to 140, the temperature rose to 101°, severe palpitation and dyspnea appeared. On October 15 and again on October 16, digitan, 1.0 gram was administered. The heart rate now dropped promptly, the temperature fell, and the weight decreased. Quinidine was given again on October 24 and 25, but once more tachycardia, gain in weight and fever followed, relieved by the exhibition of digitan on the following day. The use of quinidine had twice
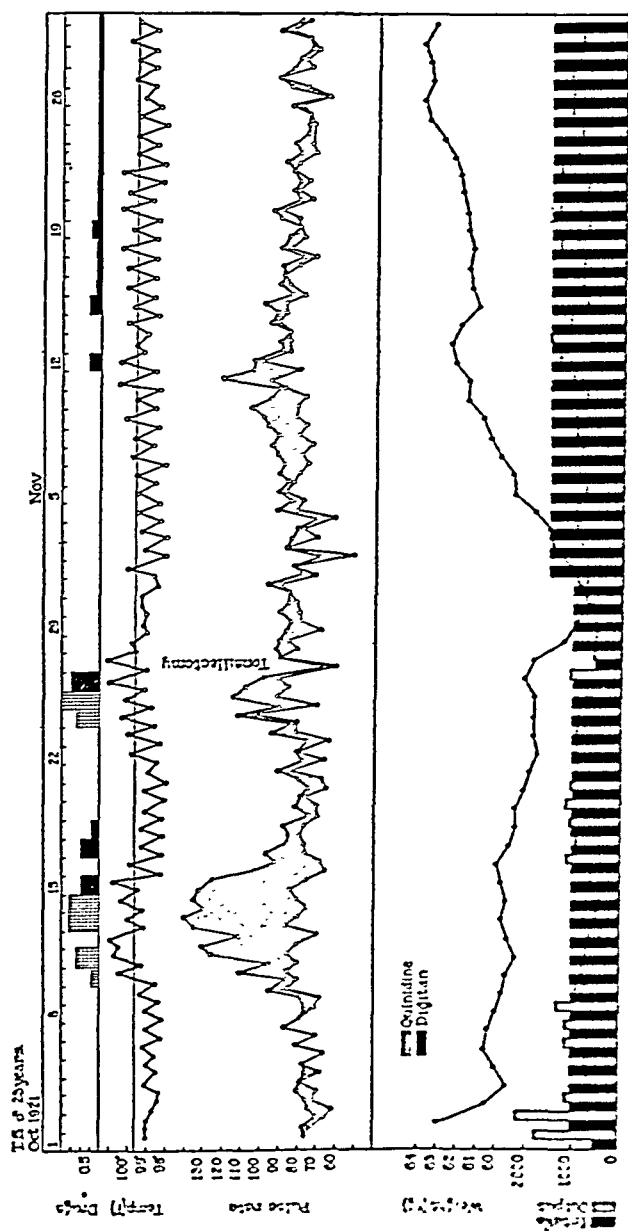
FIG. 4. IN THE CHART OF CASE IV, FEVER AND TACHYCARDIA FOLLOW THE ADMINISTRATION OF QUINIDINE ON TWO OCCASIONS

failed to restore the normal rhythm. Tonsillectomy was performed October 26. Immediate recovery from the operation was uneventful, but the heart rate and the weight increased slowly. On November 11 fever appeared. All these symptoms were relieved by giving digitan.

The remainder of the patient's illness may be told briefly. He was readmitted in September 1922, November 1923 and October 1925. Each time the degree of heart failure was more severe and recovery more difficult. He was admitted for the last time in January 1926. For a while, improvement took place, but on March 9, three days after the development of bronchopneumonia with high fever, he died.

Postmortem examination confirmed the presence of a bronchopneumonia. The heart was large, adherent fibrous pericarditis was present. The mitral valve was stenosed to a marked degree by a heavily calcified ring. The other valves were normal. Numerous fibrous scars of the myocardium were found. Chronic passive congestion of the liver was present.

Case V. R. S., Hospital Number 4144, female, aged 50 years, was first admitted on April 1, 1920, complaining of palpitation of sudden onset nine weeks before, followed by extreme weakness. A history of rheumatic fever, chorea, sore throats or colds was not obtained. She was told at age 16 of the existence of serious heart trouble but had experienced no difficulty. Dyspnea, edema and precordial pain had not occurred. Digitalis in some form had, however, been administered occasionally. At 45 she suffered from an attack of influenza followed by sinusitis. With the exception of those due to her cardiovascular system there were no abnormal physical signs. The heart was considerably enlarged. Diastolic and systolic murmurs were heard at base and apex; the totally irregular rhythm was due to auricular fibrillation. No signs of congestion were present. The blood pressure measured 164 mm. Hg systolic and 86 diastolic. Obvious foci of infection were not found. The tonsils were small and atrophic, the sinuses clear, but a few carious teeth were present. Examination of the urine was negative. The Wassermann reaction of the blood was negative.

At first, cough was her most prominent symptom and was associated with increase in dyspnea, slight enlargement of the liver, pulmonary congestion and moderate increase of the cardiac rate. All these symptoms were relieved by taking digitalis. There was occasional elevation of temperature usually associated with increase in the degree of heart failure. On one occasion it rose to 101° in association with an attack of paroxysmal tachycardia. She was discharged August 21, 1920.

A few months later, October 24, 1920, she was readmitted in heart failure of moderate degree. The cardiac signs were unchanged. The use of digitalis relieved her symptoms. On January 6, 1921, she suffered an acute upper respiratory infection with cough, sore throat and hoarseness, while taking digitalis (Fig. 5a). She was free of edema or pulmonary congestion, but fever, tachycardia, and loss of weight were present. She recovered in 3 or 4 days. On February 26, the administration of digitan was discontinued. Shortly thereafter the heart rate began to increase. By March 17, dyspnea, cough and palpitation had become severe, slight edema of the shins had appeared and the liver had descended from 3 cm. to 8 cm. below the costal margin. Instead of loss in weight, a slight increase occurred. Fever was present until, with the administration of digitan on March 19, improvement began (Fig. 5b). During the latter part of April fever accompanying another acute upper respiratory infection occurred (Fig. 5c). At this time she was receiving digitan 0.1 gram a
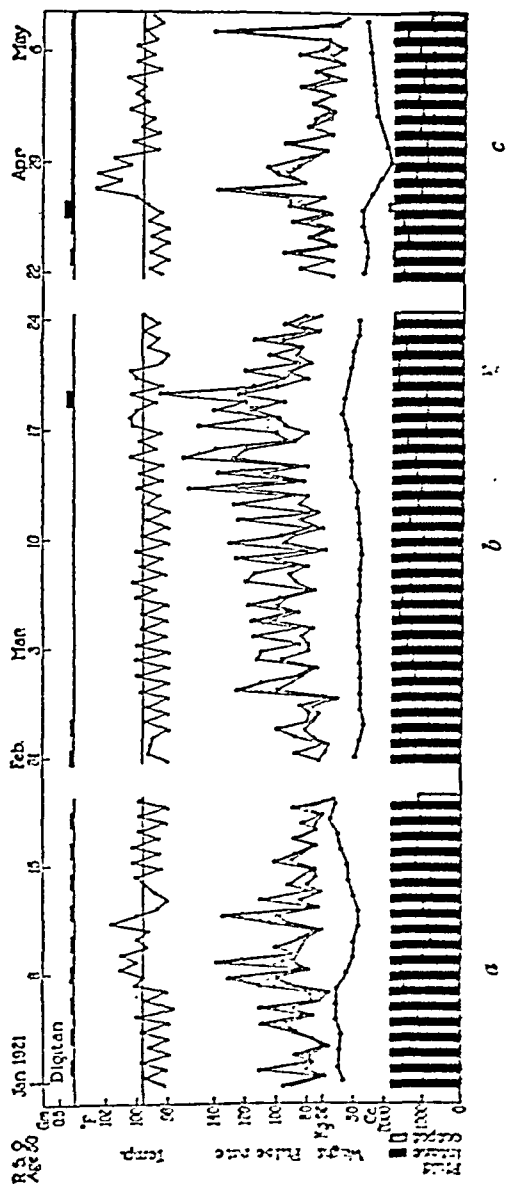
FIG. 5. THE CHART OF CASE V ILLUSTRATES THE BEHAVIOR OF PULSE RATE, TEMPERATURE AND WEIGHT (a) DURING AN ACUTE UPPER RESPIRATORY INFECTION WHILE THE PATIENT IS RECEIVING DIGITALIS, (b) DURING THE PERIOD OF OMISSION OF DIGITALIS AND (c) DURING A MORE SEVERE ACUTE UPPER RESPIRATORY INFECTION.

Nine postmortem examinations were performed. Blood cultures made in all the patients before death were negative. In five instances pulmonary infarcts were found. These were sterile except one from which *B. coli* and a gram-positive bacillus were cultivated. Direct cultures of pulmonary tissue were made. In seven of the nine cases material obtained both antemortem and postmortem was sterile. In the eighth case *Bacteriodes acuminatus* obtained before death by lung puncture, was obtained postmortem also from the lungs, the heart's blood and the pleural fluid. In the ninth case postmortem one lung contained *Staphylococcus hemolyticus* and the other was sterile. Material obtained before death by puncture of the lungs was sterile. From these experiences there appears no reason for regarding the lungs as infected.

These studies recall Thacher's (6) observations concerning fever in heart disease. In 1905 he commented on autopsies of a large number (901) of cases of chronic endocarditis, many (505) of which exhibited fever though its cause in more than half (291) was obscure. Exacerbation of the rheumatic process would doubtless be assigned now as the most likely explanation of its occurrence. But Thacher felt forced to remark somewhat forlornly that "the autopsy as well as the clinical picture awarded nothing which would have been apt to produce fever unless simple passive congestion of the viscera can do so."

Clearly, however extensive the search for an infectious source, failure to find it does not assure its absence. On the other hand that the train of events itself in heart failure may lead to the occurrence of fever is an idea which has occasionally been entertained, as the quotation from Thacher shows. In fact von Bärensprung (7) (1852) ventured to suggest that fever accompanied hypertrophy of both ventricles, or particularly that of the left when the volume of the pulse was great, while subnormal temperatures occurred when the pulse was small and the right ventricle dilated.

There is evidence, furthermore, of a different nature that fever may be circulatory in origin. Following death, when the circulation has ceased the temperature of the body sometimes continues to rise. Wunderlich (1) (p. 287) calls attention to Seume's (8) first having observed (1856) this phenomenon and to his own confirmation of this observation. In studying further the mechanism underlying this idea Heidenhain (9) demonstrated (1870) in dogs that stopping the respiration resulted in a fall of temperature while cessation of the heart beat brought about a rise and he noticed also that compression of the thoracic aorta was followed by a rise in temperature of the liver and intestinal contents. A few years later Ackermann (10) showed, also in dogs, that rise of the internal temperature immediately after death took place while the peripheral temperature was continually falling. Since the blood pressure was zero, and since artificial respiration was still in progress the rise in rectal temperature *could*

be attributed only to cessation of the circulation. Based likewise on the idea that level of temperature is modified by conveyance away by the blood stream of heat locally developed, Binger and Christie (11) have more recently shown that in lungs in which the circulation is stopped local temperature rises promptly with the introduction of artificial heat (diathermy). Nothing has been found in the literature to indicate, however, to what degree this phenomenon occurs when the circulation is slowed rather than stopped. In a similar sense, also with regard to the transportation of heat to the periphery, elevation of the temperature occurs in "sympathetic fever" (12), after the administration of cocaine (13) and after puncture of the corpus striatum (14), due apparently to diverting the circulation of blood in large measure from the surface of the body.

In heart failure when the circulation as a whole, to the skin as well as to other parts of the body, is slowed, it seems likely, accordingly, that elevation of the temperature in the interior parts of the body may occur. But it is quite possible that the sequence of events leading to the accumulation of heat is not so direct: the so-called heat centers of the brain may be incapacitated through faulty circulation and may fail to regulate, through faulty vasomotor arrangements at the surface of the body, the loss of heat from the skin. Another possible origin of fever—chemical in nature—is the formation in the tissues, due to lack of oxygen, of a substance toxic in nature and capable of inducing fever. Such substances have been found by Krehl and Matthes (15) and many others during the course of infectious diseases. Mandel (16) has in fact demonstrated the presence of such a substance in the urine of patients suffering with aseptic (surgical) fevers.

A discussion on the ultimate mechanism involved in the causation of non-infectious fever is, however, beyond the scope of the present study. At the moment no more is intended than to describe a relation between heart failure and fever which in certain instances suggests dependence of the latter (fever) upon some process involved in the former (heart-failure).

SUMMARY

1. Fever may occur during heart failure in the absence of evidence of infection or of the non-infectious conditions which have been enumerated and which are likewise associated with the development of fever.

2. Results of bacteriologic studies of material obtained by puncture of the lungs during life and from the lungs at autopsy in patients with heart failure accompanied by fever are presented.

3. In a number of cases signs of heart failure appear or begin to increase just prior to the occurrence of fever; fever and the signs of heart failure disappear simultaneously.

4. These relations suggest that the occurrence of fever in these instances is dependent upon the mechanisms involved in heart failure itself.

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FEVER IN HEART FAILURE

RELATIONS BETWEEN THE TEMPERATURES OF THE INTERIOR AND THE SURFACE OF THE BODY

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Fever has for a long time been observed to be a very frequent symptom during heart failure. Often the elevation of temperature is associated with infectious processes, often with non-infectious ones as, for example, infarction of various organs, cerebral hemorrhage or hyperthyroidism. Many times meticulous search fails, however, to bring to light any of these conditions. It has then been generally assumed that one of the more familiar processes is present but has escaped detection. In lungs already the seat of chronic passive congestion bronchopneumonia might easily remain undiscovered.

Fever during the course of heart failure has remained inexplicable so frequently that several observers have developed hypotheses to account for its occurrence. Cohn and Steele (1) reviewed these briefly and supported with clinical evidence the idea that heart failure itself may lead to the elevation of rectal temperature. The evidence consists in having related in point of time the appearance of the signs of heart failure with the appearance of fever when no recognized causes of fever are discoverable.

It is a venerable observation that people with " poor circulation " have cold hands and feet while the extremities of patients with infectious diseases possessing presumably normal circulations are hot. This important difference suggests the desirability of studying the relations between the temperature of the surface and that of the interior of the body. Changes in the conditions of the environment undoubtedly influence this relation and must therefore be kept under control or, in any event, carefully recorded. Calorimetry did not appear to be the best method to employ in this study since the problem concerns not a discrepancy between heat produced and heat lost but the mechanism by which thermal equilibrium is maintained or established within the body under conditions which differ from those encountered in normal individuals.

Knowledge of the variations in temperature of the surface in conse-

quence of bodily activity and of changes in environment has been contributed by so many observers that a complete list of citations has not been attempted. Benedict perhaps has been responsible, more than any other individual, for arousing interest in the subject. Together with a number of collaborators he has shown that, although much more variable than internal temperature, the temperature of the surface is fairly constant and uniform when the environmental conditions are adjusted so as to meet the subject's requirements for personal comfort. He has found that the trunk varies usually between 33.5 and 36.5° C. (2). The temperature of the extremities is not nearly so constant. When the environmental temperature is approximately 25° C. it appears though that, in subjects at rest and comfortable, the surface of the extremities too, is fairly uniform. In 12 girls the surface temperatures ranged from 30.3° C. to 35.2° C. and in four women from 33.2° C. to 35.5° C. At rest the average temperature of the skin is about 34° C. (3). With Parmenter he has shown that the surface temperature falls with fall in environmental temperature. This fact has been confirmed by many observers, notably by Talbot (4) who confirms also the fact that the immediate effect of exercise is to lower the surface temperature. Talbot makes clear that the temperature of the extremities varies to a greater extent with the temperature of the environment than does that of the trunk. He observed that the usual range of relative humidity has little if any effect. Foged (5) gives the range of temperatures of the abdominal skin as 33.6 to 36.9° C. These figures agree fairly closely with those of Benedict and were obtained from records of more than 2700 observations in normal people. The feet he finds extremely variable. The minimum found in normal individuals was 22.7° C., the maximum 34.3° C. Foged emphasizes the important observation that symmetrically located points on the surface of the body are at the same temperature. Symmetrical points on the two sides of the body differ by 0.5° C. or less in 75 per cent of his observations. The greatest difference noted is 1.2° C. Ward (6) correlated sensations of comfort with surface temperature in the region of the carotid artery and on the forehead and her figures fall within the range found by Benedict.

A very interesting diurnal variation has been reported by Kirk (7). He noticed that in all except debilitated individuals the temperature of the feet rose each night just before the onset of sleep in a fashion quite similar to that noted by Ipsen (8) during the initial stages of anesthesia. The reaction to anesthesia has since been used extensively as a test for distinguishing local circulatory disorders due to structural arterial disease from those due to " spasm " of the vessels (9, 10). Gibbon and Landis (11) have more recently shown that simply by immersing one or more extremities in water at 45° C., excellent dilation of the vessels in the extremities not so treated may be obtained. This was an extension of the method of Lewis and Pickering (12) of obtaining peripheral dilatation by heating

the whole body. All of these reactions serve to emphasize the potential variability of the temperature of feet and hands.

From the foregoing paragraphs it appears that in spite of the accumulation of considerable data on factors which influence the temperature of the skin and the extent to which temperature may change under various circumstances, a clear conception is still lacking of the extent and form of the ordinary diurnal variation of many points on the skin of a normal individual at rest. In view of the variability of surface temperatures, knowledge of the form and extent of the usual daily variations is necessary for describing their unusual variations. The study of normal individuals as well as of cardiac patients at rest under a set of uniform environmental conditions and over periods of observation lasting at least twenty-four hours was, therefore, undertaken.

A description of the equipment and procedure used follows:

1. A room of approximately constant cooling power was employed in which the temperature was maintained between 20.5° and 21.5° C., the relative humidity between 40 and 50 per cent and the rate of movement of air between 2 and 3 meters per second. The sum of the effect of these three factors was measured at intervals by means of Hill's kata-thermometer. The resultant cooling power varied between 4.0 and 4.4 (dry) and between 15 and 16 (wet) millecalories per square centimeter per second.

2. Surface temperatures were measured by means of copper-constantan thermocouples. In a few of the earlier observations a single thermocouple was employed. Considerable disturbance of the individual under observation was entailed in measuring, by a single exploring thermal junction. many points scattered widely over the body. Before continuing the study, therefore. ten thermocouples were made and adjusted so that they could be held in place throughout the period of observation. The units were made as nearly similar as possible. Number 30 gauge wire was used for both copper and constantan elements. The constantan wire was carefully tested for its uniformity of composition by immersing the whole length, a foot at a time, in hot water while the ends were connected to a galvanometer. Each unit was adjusted to the same sensitivity by altering the length of the constantan element. The junctions to be kept at constant temperature were placed in a bath for the purpose described by Clark (13). Some difficulty was experienced in obtaining thermocouples with the same zero point. The differences were due partly to the type of temperature bath used and partly to the switches and circuit connections. Correction involved, however, only simple addition to or subtraction from the reading obtained of 0.1° or, in one instance 0.2° C. The copper poles of the thermoelectric units were connected by telephone cable (16 gauge copper wire) to a two-way ten-point rotary switch (Leeds and Northrup) by means of which any thermocouple could be quickly connected to the galvanometer. The switches, galvanometer, lamp and scale were located in an adjoining room. The galvanometer was a Type R. Leeds and Northrup moving coil suspension of 12 ohms resistance with a balistic period of 7 seconds. A variable resistance was introduced in order that the sensitivity could be so adjusted that 1 cm. deflection of the beam of light was equal to 1° C. change in temperature. A change of 0.1° C. was therefore easily read.

The apparatus was calibrated in a well stirred water bath with a Bureau of Standards thermometer. Under these circumstances subsequent readings of the temperature of the water by means of the thermocouple did not differ from that read on the standard thermometer by more than $\pm 0.025°$ C. The accuracy of thermocouples as applied to measurements of surface temperature will be considered later.

The procedure adopted for a period of study was as follows. The subject was placed in the room of constant cooling power, in bed, at 8 A.M., one to one and one-half hours before measurements were begun. The thermal junctions were made fast to the skin by adhesive tape at 10 points (Fig. 1). One
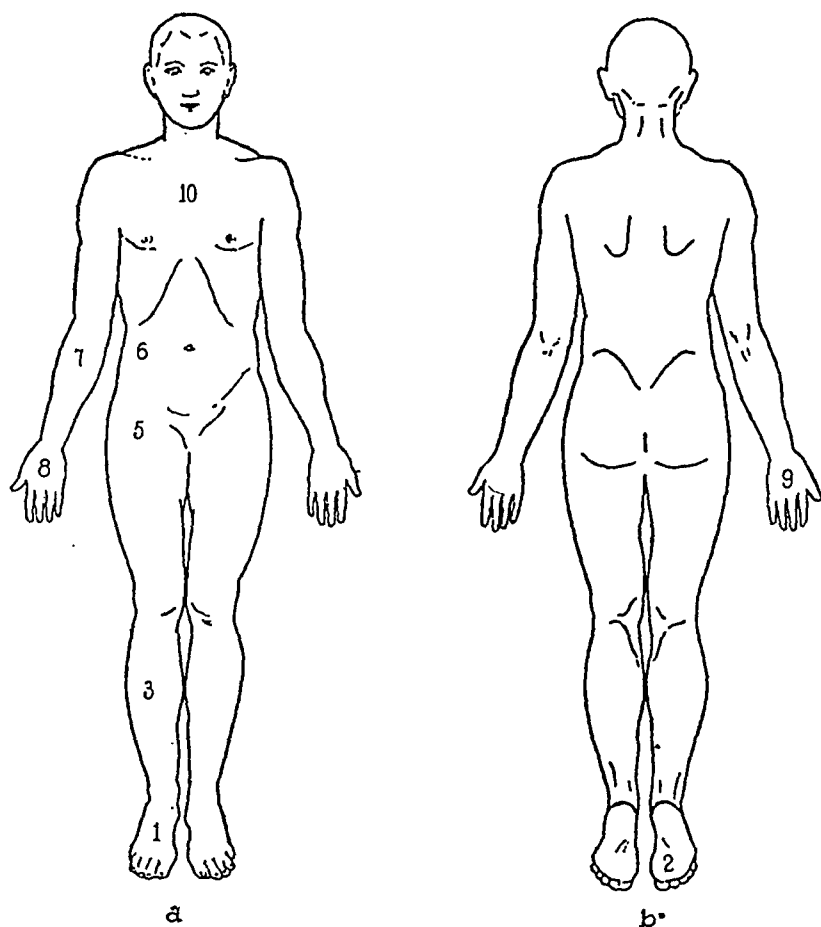
FIG. 1. OUTLINE DRAWINGS ARE SHOWN OF THE ANTERIOR (a) AND POSTERIOR (b) SURFACES OF THE BODY TO SHOW POSITION AT WHICH THERMOCOUPLES WERE PLACED.

side only of the body was studied because of Foged's (5) observations on the similarity of temperature of the two sides. The patient enjoyed much greater freedom by use of this procedure. The temperature of the surface at points on the left side similarly placed to those on the right were taken, however, at the beginning of each experiment. Unusual differences between the two sides were not encountered. The thermal junctions were mounted on bits of cork measuring approximately 10 x 5 x 3 millimeters which prevented the adhesive

tape from touching the skin in the region immediately adjacent to the thermocouple. A thermal junction encased in a rubber catheter was inserted to a depth of 10 centimeters into the rectum; one enclosed in a cage with vents for circulation of air was laid at the foot of the bed beneath the covers.

Since the study was conducted on patients, some of whom were seriously ill, it was impossible to deprive them over a long period of time of clothing. Every effort was expended, however, to see that the coverings employed were similar. The usual cotton pajamas of the hospital were worn. The bed clothes were always identical and consisted in (1) a standard hospital sheet and (2) a bedspread between which (3) one of two medium-weight wool blankets, selected because of their similarity in weight and texture, was placed. The diet consisted of 1800 calories with relatively little protein. The temperature of food and fluid was not greater than body temperature nor less than room temperature. Baths were omitted. The hands and face were cleansed quickly with a cloth dampened with water at room temperature and quickly dried. This was always done immediately after the 8 A.M. and 5:30 P.M. records were obtained. Activity was limited to reading, but no effort was made to maintain any particular position. Change of position, unusual exposure of a part of the body, placing of the hands under the covers, eating, sleeping, waking and any unusual occurrences were recorded in the nurse's notes. Unless specifically mentioned drugs were not given immediately before or during the period of observation.

Readings of the temperatures at the thermal junctions were taken every half-hour, occasionally in cases of rapid change, every quarter of an hour. Readings of the wet and dry bulb thermometers were recorded every two hours. In addition a continuous record of room temperature was obtained by means of a Tycos recording thermometer. The rate of consumption of oxygen was measured by means of a Benedict-Roth apparatus before breakfast on the morning on which the test ended in order that the rate of heat production at the time of the test might be estimated.

The records of the individuals studied are summarized in Tables I and II.

RESULTS

Few facts of diurnal variation in biology are more frequently recorded or better known than the regular daily fluctuation of internal, usually rectal or oral, temperature in man. From the data which are the subject of the present report it is apparent that under constant environmental conditions the surface of the body too, is subject to a fairly regular diurnal variation in temperature but that it is more easily influenced by the conditions of the environment. When the subject is at rest in bed in a room with an environmental temperature of about 20.5° to 21.5° C. variations somewhat as follows take place. The temperature of the surface of the trunk changes least, that of the extremities most. The direction of change in temperature of the extremities is in general opposite to that of the rectum; [1]

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[1] The same results have been obtained by Heiser, F., and Cohen, L. H., who have published a composite curve of a number of individuals each of whom was studied over a long period of time. J. Industrial Hyg., 1933, 15, 243. Diurnal variations of skin temperature.

TABLE I

Tabular summary of the records of patients with heart failure and fever *

	Case IX, 65 yrs., Male		Case X, 34 yrs., Male				Case XI, 53 yrs., Female				Case XII, 56 yrs., Female		Case XIII, 70 yrs., Female		Case XIV, 48 yrs., Male	
	Adm. 2/25/31	Disch. 6/19/31 None	1st Adm. 1/5/29	Disch. 1/26/30	2nd Adm. 5/1/31	Disch. 7/26/31 None	1st Adm. 4/11/32	Disch. 6/3/32	2nd Adm. 2/26/33	Disch. 6/2/33 None	Adm. 10/2/33	Disch. 12/22/33 None	Adm. 11/2/33	Disch. 12/28/33 None	Adm. 2/5/34	Disch. 2/7/34 None
Rheumatic infections											Sore throats till 1922. Polyarthritis July & Sept. 1922. Tonsillectomy Oct. 1922. Mitral stenosis found July 1922					
Other history	Excellent health		Heart lesion discovered in 1905				Nervous for 10 years						Pneumonia 1918		Nervousness 5 years	
Present illness — Date of appearance:	Dec. 1930		1908 (for 6 mos.) and 1924 recurred				3/1/32				July 22 on effort; Aug. 1932		11/1/33		Aug. 1931	
Dyspnea	Sept. 1930		1924				0				0		10/29/33		Aug. 1931	
Palpitation	2/18/31		0								May 1933		0		0	
Precordial pain	Jan. 1931		0				3/15/32				0		0			
Cough			9/5/29				3/1/32				Aug. 1933		10/29/33		Sept. 1931, 3 weeks before thyroidectomy; 6 weeks after thyroidectomy	
Edema			10/20/29													
Other	Ascites 2/18/31						Fainting spells from 3/1/32 to 4/15/32				Sudden attack of suffocation and palpitation 1933		Sudden attack of choking and palpitation 11/1/33			
Physical examination — Temperature, °F.	101	99	101	99.8	100.8	99.6	99.5	99.6	100.6	99.5	100.5	99.2	100.8	99.8	100.4	99.6
Pulse †	150/100	88/82	64/60	74/71	140/75	110/70	118	95	164/112	90	160/112	90/80	140	85	80	80
Blood pressure ‡	110/80	120/65	120/80		110/90	110/70	128/76	118/70	112/90	118/80	150/110	135/85	146/90	124/60	145/95	128/70
Dyspnea	+++	0	+++	0	++++	++++	+	0	++++	0	+++	0	++	0	+	0
Cyanosis	+++	0	+++	0	++++	++++	0	0	+++	0	++	0	0	0	0	+?
Edema	+++	0	+++	0	+++++	+++++	+++	0	++++	0	+++	0	0	0	0	0
Râles in lungs	+++	+	+++	++	+++++	+++++	++	?	+++	0	+++	++	++	0	0	0
Enlarged liver	+++	++	+++	+	+++++	+++++	++		+++	0	++		+	0	+	0
Enlarged heart	+++	+++	+++	++	+++++	+++++										
Murmurs	Systolic at apex		Systolic and diastolic at apex				Blowing systolic at base				Systolic at base	Systolic at base	Systolic at apex			
Rhythm	Aur. fib.		Auricular fibrillation				Normal		Aur. fib.		Aur. fib.		Aur. flutter, Dilatation & sclerosis		Normal	Normal
Aorta	Sclerosis and dilatation		0				Fusiform dilatation of aorta	Normal	Normal thoracic	Normal					Normal	Normal
Sclerosis of arteries	++++		0		0	+	++	++	++	++	+++		Normal		0	0
Other	0		0	+	0	+	?				None				0	+0
White blood count §	12,800	8,600	13,000						7700	6,750	7,400		9,750		6,400	7,300
Basal metabolism § per cent	+18 to +28	0 to +6					+25	+10	+6	+3	+8	+5			+45 to 55	+18
Culture of lungs									Hydrothorax 0	Hydrothorax 0	Hydrothorax 0					
Wassermann reaction	neg.	neg.	neg.		neg.		neg.		neg.		neg.		neg.		neg.	
Other data							Tremor						Tremor		Tremor	

TABLE I (continued)

	Case IX 65 yrs. Male	Case X 31 yrs. Male	Case XI 53 yrs. Female	Case XII 56 yrs. Female	Case XIII 70 yrs. Female	Case XIV 48 yrs. Male
Mode of disappearance of signs of congestion	Use of digitalis relieved heart failure 4 times. It recurred when drug was withheld	Prompt recovery followed rest and use of digitalis / Temporary improvement (5 days) followed use of digitalis	Prompt recovery followed 2 weeks rest in bed without use of any cardiac drugs / Improvement did not follow return to nor. rhy. on 3/3/33 but immediately after removal of 800 ccs. pleural fluid 10 days later	On 10/11/33 digitalis was given and hemorrhages from rectum occurred (2,500 cc. est.) Crisis diuresis followed with complete recovery	Slow and after prolonged use of digitalis. Nor. rhy. did not occur till after relief of signs of heart failure	Gradual improvement in nervousness and dyspnea after thyroidectomy
Diagnosis ... A	General arteriosclerosis	Unknown	General arteriosclerosis	Arteriosclerosis Rheumatic fever	Arteriosclerosis	Arteriosclerosis Hyperthyroidism
B	Enlarged heart Sclerosis of aorta	Enlarged heart mitral insufficiency	Sclerosis and dilatation of thoracic aorta. Enlarged heart	Enlarged heart	Enlarged heart	Enlarged heart
C	Aur. fibrillation	Auricular fibrillation	Normal rhythm Aur. fib. Nor. rhy.	Aur. fibrillation	Aur. flutter Nor.	Normal Normal
Remarks	Pulmonary infarct on May 10th without signs of heart failure. Died suddenly at home 10/6/31	Improvement with digitalis in May 1931 was very short lived. Progressive failure ending in death on 7/26/31. Autopsy showed only marked chronic passive congestion of viscera, slight thickening of one leaf of mitral valve and myocardial fibrosis	Patient well and free of signs of heart failure on 4/15/34	Patient well, free of signs of heart failure on 2/15/34		

* Adm. = admitted; Disch. = discharged; Aur. fibr. = auricular fibrillation; Nor. rhy. = normal rhythm.

† Whenever two figures are given that on the left denotes the apical rate, that on the right the rate of the radial pulse; where there is but one, it is the rate of the radial pulse.

‡ The figure on the left is the systolic, that on the right, the diastolic level of arterial pressure in mm. Hg.

§ Referred to the standards of Aub and Dubois.

Data concerning individuals used for control

Case number	Age	Sex	Condition at time of recording surface temperatures
	years		
I	33	Male	Normal
II	52	Male	Normal—convalescent neuritis of left musculo-spiral nerve
III	51	Male	Normal—recovered lobar pneumonia
IV	35	Female	Normal
V	37	Female	(a) Pulmonary abscess following secondary atypical pneumonia. Temperature 101–103°·F.
			(b) Normal—five months after subsidence of fever
VI	27	Female	Rheumatic endocarditis, aortic stenosis without signs of heart failure
			(a) During attack of acute pharyngitis. Temperature 100–101° F.
			(b) One month after fever had ceased
VII	35	Male	Acute rheumatic polyarthritis. Temperature 100–101° F.
VIII	34	Female	Subject of transient attacks of complete auriculo-ventricular heart-block
			(a) Complete heart-block
			(b) Normal rhythm

as the rectal temperature rises during the early morning hours, that of the skin of the extremities falls and remains low or gradually increases during the day. At or just before the time when the rectal temperature begins to decline during the evening, the surface of the extremities rises and usually attains and holds its maximum while the rectal temperature is at its minimum (Figs. 2a (Case I) and 3b (Case V)). Even under the conditions which were imposed the extent of change of the extremities varied from person to person to a considerable degree (compare Figs. 2a and 3b). Examples of extreme exaggeration of diurnal variation have been observed to occur in a patient with heart block but without heart failure (Fig. 4, Case VIII) and in a patient with arterial hypertension.

In each individual the form of the changes in surface temperatures appears to be constant and to be reproduced with considerable regularity provided, of course, that the environmental conditions are similar. The rather unusual type of curve shown in Figure 4a (Case VIII) is reproduced almost exactly in Figure 4b three months later. Whenever normal subjects were studied under the same conditions on more than one occasion the curves were quite similar. Opportunity to study one individual who had been a night-watchman for twenty-five years presented itself. There was no marked difference in the time of maximal and minimal temperatures of either surface or rectum. The form of the record obtained was similar to that obtained from individuals working during the day.

As the temperature of the environment rises above 20° C. variation of the temperature of the extremities tends to become smaller until at about
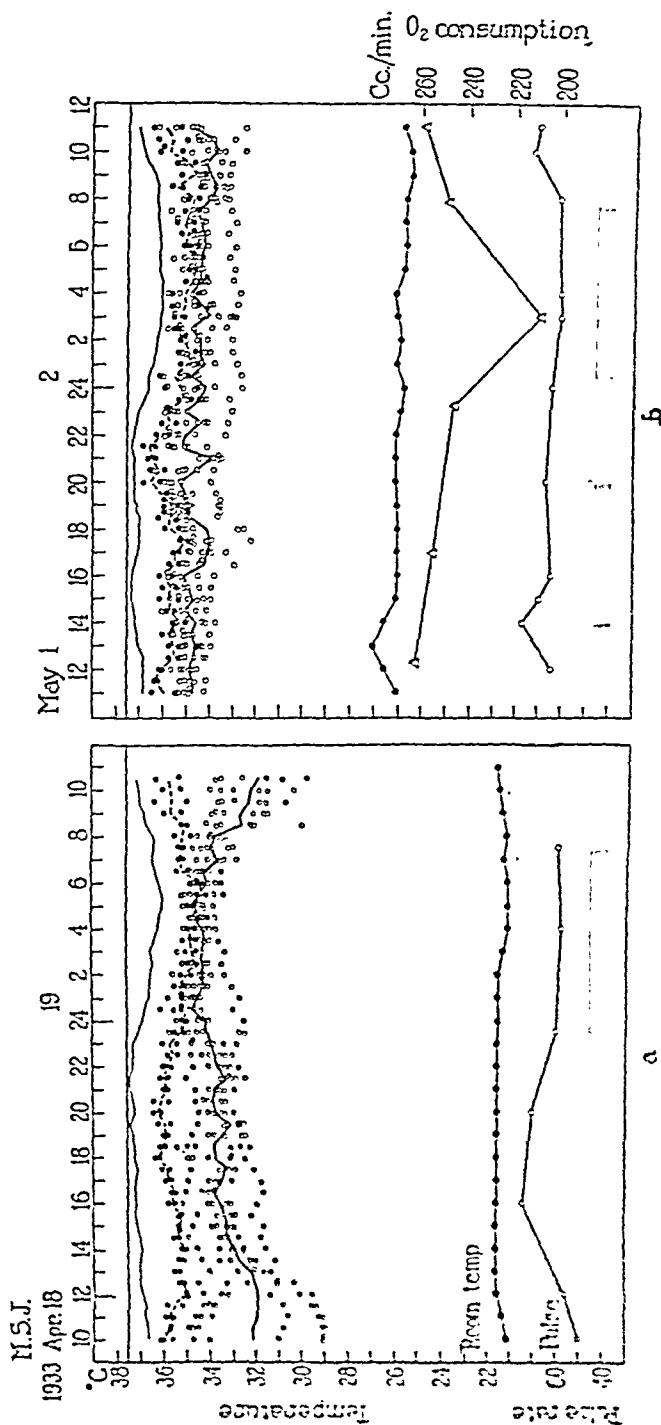
FIG. 2. CASE 1. RECORDS ARE SHOWN OF THE DIURNAL VARIATIONS OF RECTAL AND SURFACE TEMPERATURES OF A NORMAL INDIVIDUAL.

The temperature of the room was 21.5° C. (a) and 25° to 26° C. (b). In (b) the variation in temperature of the extremities has disappeared but that of the rectum remains.

In this and in the subsequent figures the hours of a day are numbered continuously from 0 to 24. The heavy solid line at the top is rectal temperature. The solid dots represent temperatures recorded at points 5 and 6 (trunk); the open dots, temperatures recorded at points 1, 2, 3, 7, 8, 9 (extremities) (see Fig. 1). The broken line connects the average temperature of points 5 and 6, the solid line, the average temperature of points 1, 2, 3, 7, 8, 9. The horizontal lines near the bottom mark the duration of periods of sleep. The open triangles connected by solid lines indicate the rate of oxygen consumption.
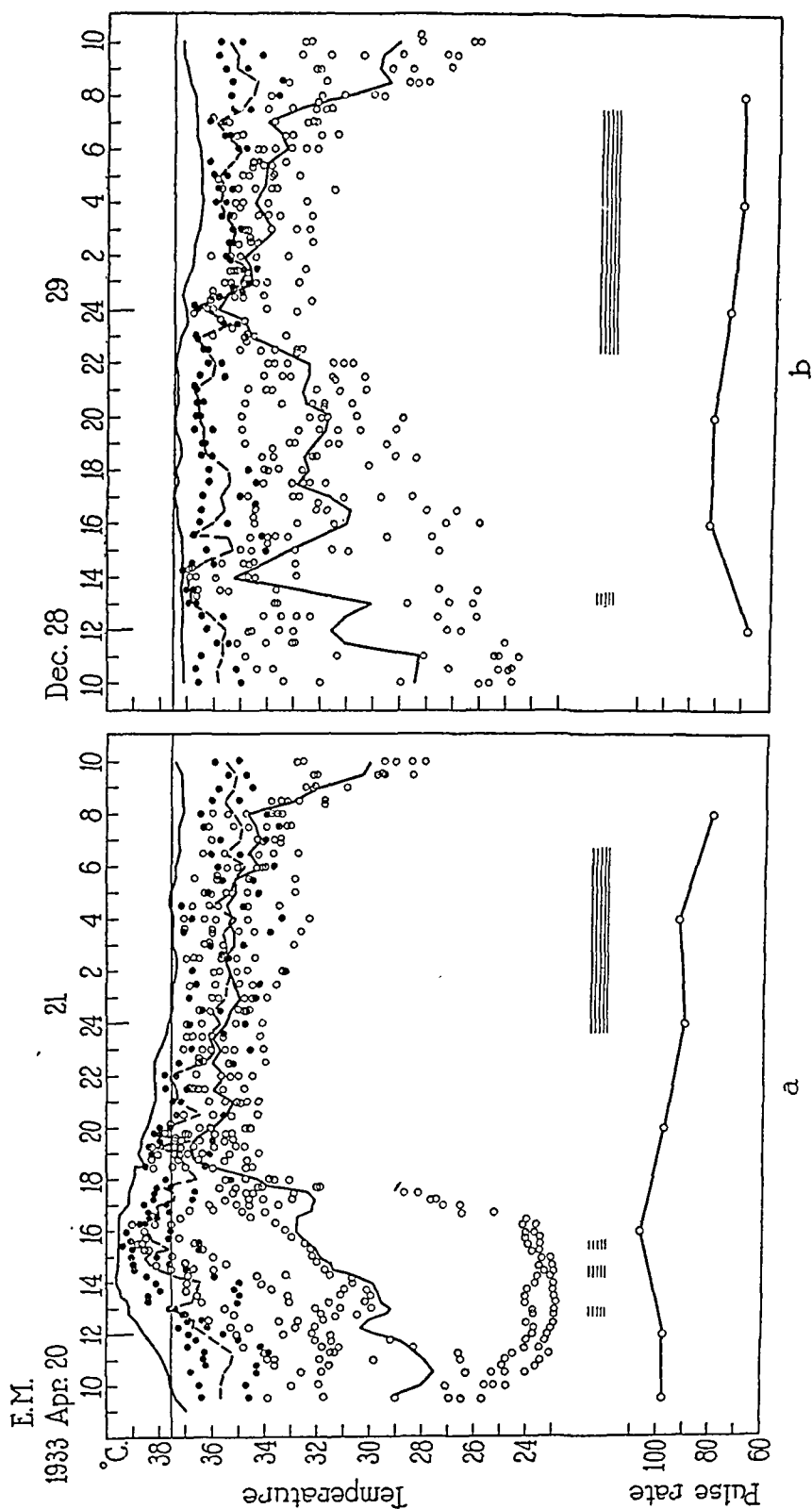
FIG. 3. CASE V. RECORDS ARE SHOWN OF DIURNAL VARIATIONS IN SURFACE AND RECTAL TEMPERATURES OF AN INDIVIDUAL (*a*) DURING AND (*b*) AFTER RECOVERY FROM PULMONARY ABSCESS WITH FEVER

In (*a*) sensations of chilliness were present (hours 14 to 17) and a mild chill with shaking occurred between 16:50 and 17:00. In (*b*) measurements taken several months later illustrate the extreme diurnal variation of the temperature of the extremities which may occur in apparently normal individuals. At 14 o'clock the influence of sleep on surface temperatures of the extremities is exhibited. In this and subsequent charts the curve of room temperature is omitted since it was always between 20.5° and 21.5° C.
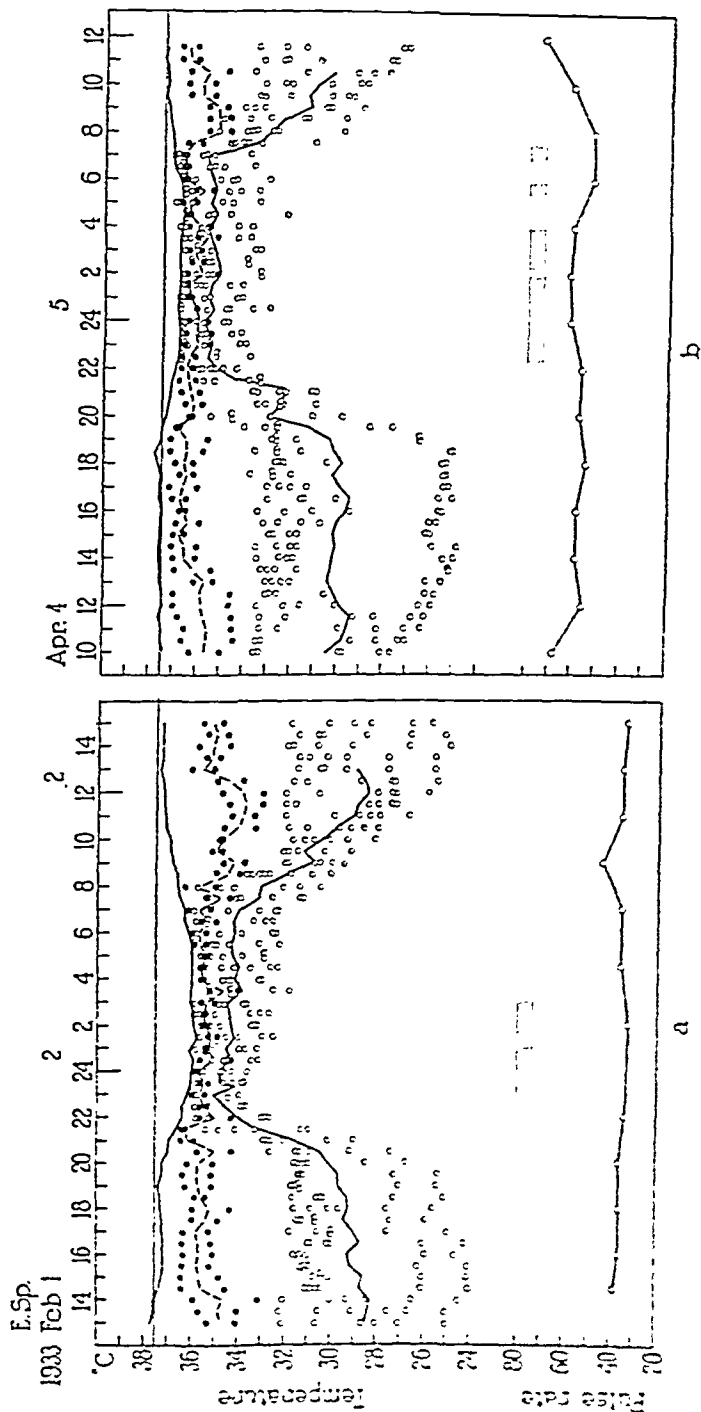
FIG. 4. CASE VIII. RECORDS ARE SHOWN WHICH WERE OBTAINED DURING THE EXISTENCE OF HEART BLOCK (a) AND DURING NORMAL RHYTHM (b) IN AN INDIVIDUAL WITH TRANSIENT ATTACKS OF COMPLETE HEART BLOCK There is noteworthy similarity between the two curves.

25° or 26° that of the surface of the body is fairly constant and uniform (Fig. 2b). The limits of variation are 32.0° and 36.4° C. and fall within the range of Benedict's (2) observations at an environmental temperature of 25° C. The greater uniformity and constancy obtained at this temperature obviously depend upon the fact that the temperature of the extremities does not at any time fall far below that of the trunk. They appear to be constantly occupied in radiating heat.

The circumstances under which increase in temperature of the extremities takes place are precisely those which Kirk (7) described. The rise in temperature was usually associated with drowsiness and preceded the moment of falling asleep by one-half to one and a half hours (Fig. 3b). The fact that in one of two instances recorded by Talbot (4) sleep was not associated with a rise in the temperature of the feet may have been due to the height of the room temperature (24° C.). At this temperature the feet are usually already too warm to exhibit an appreciable rise. Such a situation has just been referred to in the present series (Fig. 2b). Under similar circumstances even the rise which usually follows anesthesia may not occur. Lewis and Pickering (12) have pointed out that the cooler the extremity the greater is the ensuing rise in response to warming the body. Talbot's observations were made in children and the behavior of their extremities on falling asleep may be altogether irregular. In the present study it was, however, one of the more constant relationships observed both in health and disease, in natural sleep or sleep induced by sedatives, provided that the temperature of the environment was below 22° C.

In a number of individuals who were suffering from heart failure similar studies of surface and rectal temperatures were carried out. Observations were, however, either continued over a period of forty-eight hours, the latter half of which followed administration of large doses of digitalis, or repeated after patients had recovered from the attack of heart failure. The patients were selected because they had fever during attacks of heart failure and because at the time, evidence of the presence of hyperthyroidism, infarction, cerebral injury (hemorrhage or thrombosis) or infection was absent.

The *form* of diurnal variation in surface temperature obtained from individuals during *heart failure* with fever is similar to that observed in normal individuals. The relation between the *level* of diurnal variation of the interior of the body and that of the surface is, however, considerably altered. While rectal temperature is high, surface temperature is lower in heart failure than in normal subjects. The difference between surface and internal temperature is, therefore, distinctly greater than is found to be the case in normal individuals exposed to the same environmental conditions. Reduction in temperature of the surface of the extremities is marked, that of the surface of the trunk only slight (Figs. 5, 6a, Cases X, XI, respectively). The extremities remain cool for a considerably
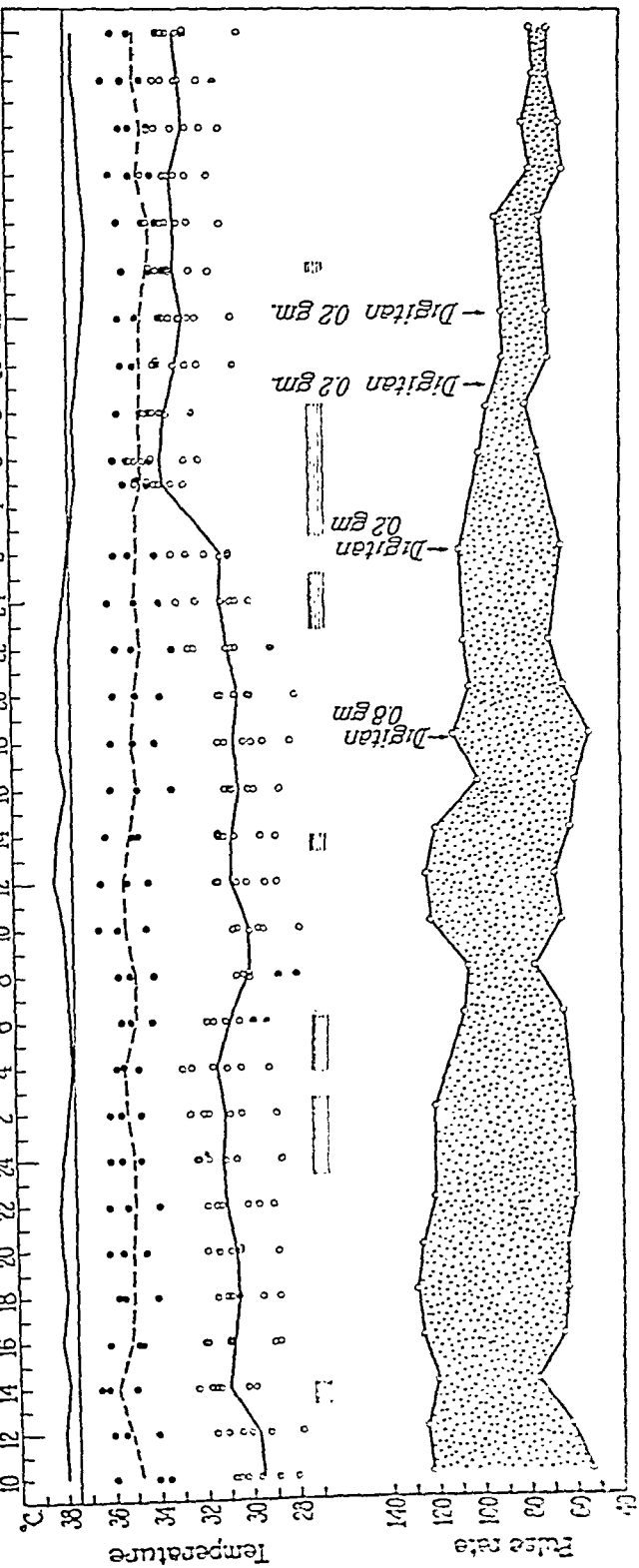
Fig. 5. Case X. A Record Is Shown Obtained from an Individual in Extreme Heart Failure with Congestion Before, During, and After the Administration of Digitalis

Surface temperatures of the extremities did not rise as high as the usual normal level at night. The stippled area represents the difference between the rates of the apical and the radial pulse.
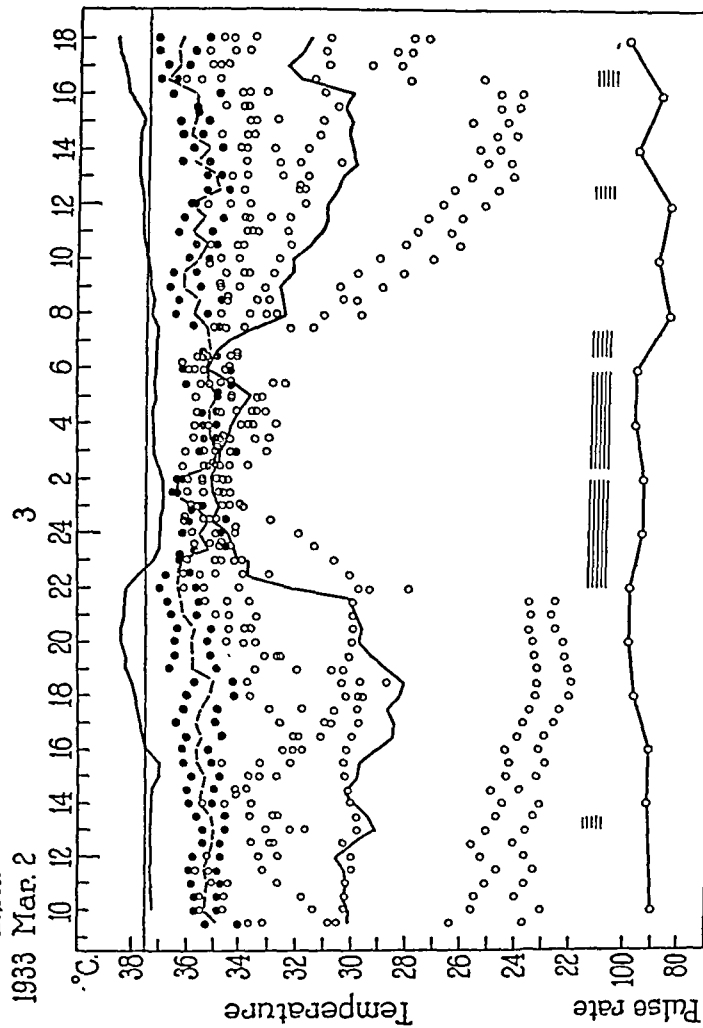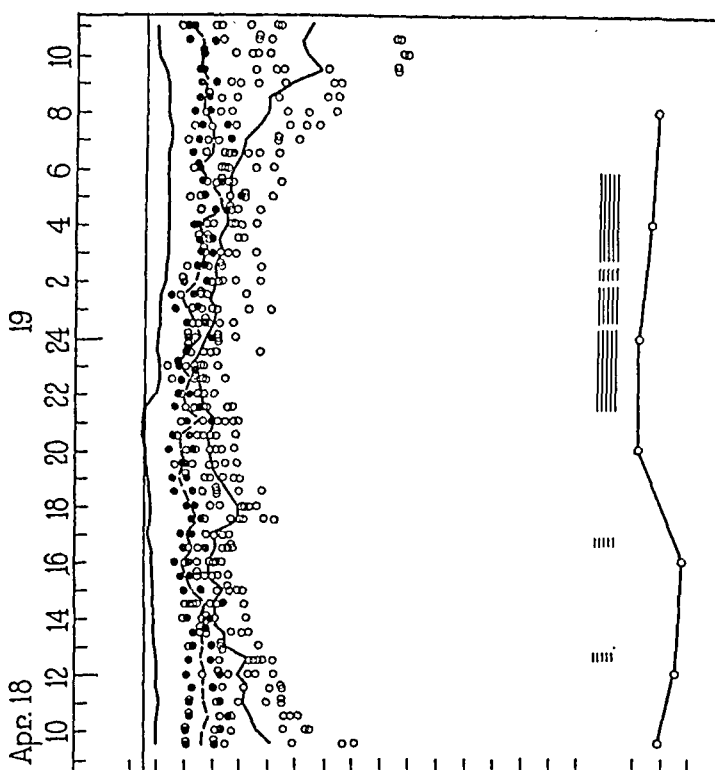
Fig. 6. Case XI. A Record Is Shown of Surface Temperatures Obtained During Heart Failure (a) and After Recovery (b) The curve of the temperature of the extremities is in marked contrast to that shown in Figure 5, in that for a short period of the twenty-four hours recorded during heart failure it rose as high as in health (b).

greater portion of the twenty-four hours than is normally the case. The average difference between rectal and surface temperatures for the whole period of twenty-four hours in patients with heart failure is accordingly distinctly greater than the average difference for the same period in normal subjects (Table III).

Data for a finer method of comparison are also available. The temperature of an individual in an attack of heart failure may be compared with that of the same individual after recovery (Figs. 5, 6, and Table IV). The fact that diurnal variations in a single individual are relatively constant if the physiologic state of the individual and the environmental conditions are the same enhances the value of this method of comparison. With recovery from heart failure accompanied by fever surface temperature rises and rectal temperature falls; diurnal variations become more like those of normal subjects.

The presence of edema is apparently not responsible for coolness of the surface of the extremities. That this is so can be inferred from the following observations. On two occasions digitalis was exhibited in the middle of a prolonged period of observation. In both instances decline of rectal and rise of surface temperature occurred before appreciable change in amount of edema, as evidenced by lack of change in body weight or in appearance, had taken place (Cases IX and X). In another instance change in temperature occurred in the absence of observable edema. The hands, moreover, were not edematous in any of the cases studied and yet the temperature of their surfaces rose with improvement of the circulatory state.

Fever of an infectious nature was studied in several individuals. The relation between internal and surface temperatures was found to be quite different from that in the group just described. If the various classes of individuals are described in terms of thermal gradient the magnitude of which is indicated by the difference between rectal and surface temperature, the following distinctions can be made. In individuals with infectious fevers the gradient is normal or less than normal. In individuals with heart failure and fever the gradient is greater than normal. The average internal temperature of both classes of cases with fever is roughly the same, the average surface temperature of those with infectious fever is higher than normal, of those with heart failure lower (Fig. 7). If the temperatures of the various points obtained at half-hour intervals are averaged for a period of twenty-four hours and a single difference between the average rectal and average surface temperatures is obtained, differences between the several classes are clear. In the group of cases studied during heart failure it was found that the difference between the average temperature of the rectum and that of the extremities for twenty-four hours (6.5° C.) was considerably greater than that found both in the normal group (3.7° C.) and in the same group after recovery from heart failure (3.8° C.). In the group

TABLE III

Temperatures of rectum, trunk, and extremities obtained for individual cases, and combined averages of each group as a whole

| Type of patient | Rectum | | | Trunk | | | Extremities | | | Differences between rectum and | | | | | |
| | | | | | | | | | | Trunk | | | Extremities | | |
	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.
Normal															
Case I	37.4	36.2	36.9	36.2	34.3	35.4	34.7	31.9	33.5	3.1	0.7	1.5	5.5	2.2	3.4
II	37.4	36.5	36.9	35.6	34.2	34.8	33.9	30.9	32.5	3.2	0.9	2.1	6.5	2.6	4.4
III	37.5	36.9	37.3	36.7	35.6	35.9	35.8	32.3	34.9	1.9	0.2	1.4	5.2	1.1	2.4
IV	37.4	36.7	37.1	35.9	34.7	35.0	34.3	29.6	33.1	2.7	0.8	2.1	6.3	2.4	4.0
V	37.5	36.5	37.1	37.0	35.0	35.8	35.9	28.2	32.6	2.5	0.1	1.3	9.3	0.6	4.5
VI	37.5	36.6	37.2	36.0	34.9	35.4	34.5	31.6	33.3	2.6	0.6	1.8	5.9	2.1	3.9
Average	37.4	36.6	37.1	36.2	34.8	35.4	34.8	30.7	33.3	2.7	0.5	1.7	6.4	1.8	3.8
Heart failure															
Case IXA	38.0	37.1	37.5	35.1	33.9	34.4	31.6	29.0	30.3	4.1	2.0	3.1	9.0	5.5	7.2
IXB	37.8	37.4	37.6	34.8	33.9	34.2	32.0	29.2	30.5	3.9	2.6	3.4	8.6	5.4	7.1
X	38.1	37.6	37.9	35.7	34.7	35.1	31.2	29.7	30.5	3.4	1.9	2.8	8.4	5.4	7.4
XI	38.4	36.9	37.4	36.5	35.0	35.6	35.1	28.2	31.7	3.4	0.4	1.8	10.2	1.8	5.7
XII	37.7	37.0	37.3	36.6	35.3	35.8	33.8	30.2	32.1	2.4	0.8	1.4	7.5	3.2	5.1
XIII	37.7	37.0	37.4	35.7	33.0	34.8	32.6	30.0	31.2	4.7	2.3	2.6	7.7	4.4	6.2
Average	37.9	37.1	37.5	35.8	34.3	35.0	32.7	29.4	31.0	3.6	1.7	2.5	8.6	4.4	6.5

TABLE III (continued)

Type of patient	Rectum			Trunk			Extremities			Differences between rectum and					
---	---	---	---	---	---	---	---	---	---	Trunk			Extremities		
	Maximum °C	Minimum °C	24 hour average °C	Maximum °C	Minimum °C	24 hour average °C	Maximum °C	Minimum °C	24 hour average °C	Maximum °C	Minimum °C	24 hour average °C	Maximum °C	Minimum °C	24 hour average °C
Heart failure—recovered															
IX	37.5	36.6	37.1	35.0	34.2	34.5	33.8	31.6	32.4	3.3	1.6	2.6	5.9	2.8	4.7
X	37.5	36.6	37.1	34.7	34.4	34.5	33.4	31.1	32.4	3.1	1.9	2.6	6.4	3.2	4.7
XI	37.6	36.6	37.0	36.3	34.8	35.6	35.7	31.2	34.5	2.2	0.6	1.4	6.4	1.3	2.5
XII	37.2	36.3	36.8	36.2	35.0	35.6	34.2	31.0	33.4	2.0	0.2	1.2	6.2	2.1	3.4
Average	37.4	36.5	37.0	35.5	34.6	35.0	34.3	31.2	33.2	2.6	1.1	1.9	6.2	2.3	3.8
Infectious fever															
Case V	39.7	37.2	37.7	38.7	35.1	36.3	36.8	27.5	33.5	3.0	0.9	1.4	12.0	1.4	4.2
VI	38.1	37.2	37.6	37.0	35.7	36.4	36.2	33.4	34.3	2.4	0.2	1.2	3.7	1.0	3.4
VII	38.3	37.0	37.8	36.9	35.4	36.1	36.5	32.9	35.2	2.9	0.1	1.7	5.4	0.5	2.6
Average	38.7	37.1	37.7	37.5	35.4	36.3	36.5	31.3	34.3	2.8	0.4	1.4	7.0	1.0	3.4
Fever of infarction															
Case IX	38.0	36.9	37.4	36.0	34.1	35.2	34.5	32.5	32.9	3.9	0.9	2.2	5.5	2.4	4.5

FIG. 7. THE CHART IS A COMPOSITE ONE OF AVERAGE VALUES OF THE GROUP OF NORMAL INDIVIDUALS AND OF THE GROUP OF PATIENTS WITH HEART FAILURE.

The solid symbols trace the average curves of the normal groups; the open ones, of the group in heart failure. Note the rise of temperature of the extremities at 13 o'clock (1 P.M.) which is the beginning of the "rest hour" in hospital routine. Although several of the patients fell asleep, it is much less marked in the group of patients with heart failure.

with infectious diseases the average difference was still less (3.4° C.) (Table III). Case VII, one of subacute rheumatic fever, exhibited such a relation and the temperature of the extremities failed to show the customary morning fall while the temperature was elevated (Fig. 8). Even

FIG. 8. CASE VII. A RECORD IS EXHIBITED OBTAINED FROM A PATIENT DURING AN ATTACK OF ACUTE POLYARTHRITIS

It is similar to Figure 2b, a normal chart secured at a much higher environmental (room) temperature.

when chills occur during the course of infectious fever coldness of the surface of the extremities which is present during rise of rectal temperature does not persist long enough to affect markedly the twenty-four hour average temperature of the surface. Case V suffered a chill while her rectal temperature was rising (Fig. 3a); the (twenty-four hour) average temperature of trunk as well as that of the extremities was higher, however, during fever than after recovery (Fig. 3b) (Table IV).

On one occasion a record of surface and rectal temperatures was obtained in a cardiac patient (Case IX) during the occurrence of fever associated with an infarct of the lungs. The average surface temperature was slightly higher than that found to exist when the patient was free of signs of heart failure and had a normal rectal temperature. During heart failure, with approximately the same degree of fever by rectum, the surface temperature was, as has been mentioned, much lower than after recovery (Table IV, Case IX).

One individual with well marked signs of hyperthyroidism was studied (Table IV, Case XIV). His basal consumption of oxygen was $+$ 56 per cent and rectal temperature was slightly elevated. The average surface temperature far from falling below that observed in the group of normal persons, remained near the upper limit of the group. It is perhaps of interest that the extremities failed to show the usual morning fall, and remained constantly above 33.9° C. The chart is similar to that of Case VII, Figure 8, one of infectious disease, as well as to that of a normal individual when the room temperature is 26° C. instead of 21° C. (Fig. 2b).

<div align="center">COMMENT</div>

Two technical matters which emerge from this study require discussion. First it is recognized that the thermocouple is not a perfect instrument for measuring surface temperatures. The fact that skin is touched at all and, in the present method, covered for a period of twenty-four hours, precludes the possibility of obtaining its temperature with accuracy due to the disturbance of the manipulations. That the skin is constantly being touched by bedclothes is not necessarily an added disadvantage. Cobet (14) from comparison of temperatures of skin calculated by measuring the radiation of heat from a known area with those measured directly by means of thermocouples, believes that the latter may be too low. His measurements agree, however, fairly well with Kunkel's (15) obtained by thermocouple. He attributes the lower results of a number of other observers to the fact that the thermocouple is not well enough protected from the influence of room air to prevent the registration of a temperature intermediate between that of the skin and that of the air. McGlone and Barett (16) have, in a very thorough manner, shown that a large error from this source is, however, unlikely since in still room air at about 21.5° C. the

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when chills occur during the course of infectious fever coldness of the surface of the extremities which is present during rise of rectal temperature does not persist long enough to affect markedly the twenty-four hour average temperature of the surface. Case V suffered a chill while her rectal temperature was rising (Fig. 3a); the (twenty-four hour) average temperature of trunk as well as that of the extremities was higher, however, during fever than after recovery (Fig. 3b) (Table IV).

On one occasion a record of surface and rectal temperatures was obtained in a cardiac patient (Case IX) during the occurrence of fever associated with an infarct of the lungs. The average surface temperature was slightly higher than that found to exist when the patient was free of signs of heart failure and had a normal rectal temperature. During heart failure, with approximately the same degree of fever by rectum, the surface temperature was, as has been mentioned, much lower than after recovery (Table IV, Case IX).

One individual with well marked signs of hyperthyroidism was studied (Table IV, Case XIV). His basal consumption of oxygen was + 56 per cent and rectal temperature was slightly elevated. The average surface temperature far from falling below that observed in the group of normal persons, remained near the upper limit of the group. It is perhaps of interest that the extremities failed to show the usual morning fall, and remained constantly above 33.9° C. The chart is similar to that of Case VII, Figure 8, one of infectious disease, as well as to that of a normal individual when the room temperature is 26° C. instead of 21° C. (Fig. 2b).

<div align="center">COMMENT</div>

Two technical matters which emerge from this study require discussion. First it is recognized that the thermocouple is not a perfect instrument for measuring surface temperatures. The fact that skin is touched at all and, in the present method, covered for a period of twenty-four hours, precludes the possibility of obtaining its temperature with accuracy due to the disturbance of the manipulations. That the skin is constantly being touched by bedclothes is not necessarily an added disadvantage. Cobet (14) from comparison of temperatures of skin calculated by measuring the radiation of heat from a known area with those measured directly by means of thermocouples, believes that the latter may be too low. His measurements agree, however, fairly well with Kunkel's (15) obtained by thermocouple. He attributes the lower results of a number of other observers to the fact that the thermocouple is not well enough protected from the influence of room air to prevent the registration of a temperature intermediate between that of the skin and that of the air. McGlone and Bazett (16) have, in a very thorough manner, shown that a large error from this source is, however, unlikely since in still room air at about 21.5° C. the

TABLE IV

Average temperatures of rectum, trunk, and extremities in all cases studied both during fever and after recovery

Case number	Rectum			Trunk			Extremities			Differences between rectum and Trunk			Differences between rectum and Extremities		
	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.
Case IX—Heart failure 1a.......	38.0	37.1	37.5	35.1	33.9	34.4	31.6	29.0	30.3	4.1	2.0	3.1	9.0	5.5	7.2
1b*.......	37.6	36.5	37.1	34.9	34.1	34.3	32.8	29.1	31.1	3.5	1.6	2.8	8.5	3.7	6.0
2a.......	37.8	37.4	37.6	35.1	33.9	34.4	31.6	29.0	30.3	3.9	2.3	3.4	8.8	5.8	7.3
2b*.......	37.6	36.5	37.1	34.9	34.1	34.3	32.8	29.1	31.1	3.5	1.6	2.8	8.5	3.7	6.0
Recovered................	37.5	36.6	37.1	35.0	34.2	34.5	33.8	31.6	32.4	3.3	1.6	2.6	5.9	2.8	4.7
Infarct................	38.0	36.9	37.4	36.0	34.1	35.2	34.5	32.5	32.9	3.9	0.9	2.2	5.5	2.4	4.5
Case X—Heart failure........	38.1	37.6	37.9	35.7	34.7	35.1	31.2	29.7	30.5	3.4	1.9	2.8	8.4	5.4	7.4
Recovered................	37.5	36.6	37.1	34.7	34.4	34.5	33.4	31.1	32.4	3.1	1.9	2.6	6.4	3.2	4.7
Case XI—Heart failure........	38.4	36.9	37.4	36.5	35.0	35.6	35.1	28.2	31.7	3.4	0.4	1.8	10.2	1.8	5.7
Recovered................	37.6	36.6	37.0	36.3	34.8	35.6	35.7	31.2	34.5	2.2	0.6	1.4	6.4	1.3	2.5
Case XII—Heart failure........	37.7	37.0	37.3	36.6	35.3	35.8	33.8	30.2	32.1	2.4	0.8	1.4	7.5	3.2	5.1
Recovered................	37.2	36.3	36.8	36.2	35.0	35.6	34.2	31.0	33.4	2.0	0.2	1.2	6.2	2.1	3.4
Case V—Infectious fever........	39.7	37.2	37.7	38.7	35.1	36.3	36.8	27.5	33.5	3.0	0.9	1.4	12.0	1.4	4.2
Normal................	37.5	36.5	37.1	37.0	35.0	35.8	35.9	28.2	32.6	2.5	0.1	1.3	9.3	0.6	4.5
Case VI—Infectious fever........	38.1	37.2	37.6	37.0	35.7	36.4	36.2	33.4	34.3	2.4	0.2	1.2	3.7	1.0	3.4
Normal................	37.5	36.6	37.2	36.0	34.9	35.4	34.5	31.6	33.3	2.6	0.6	1.8	5.9	2.1	3.9
Case XIV—Hyperthyroidism......	37.8	37.0	37.2	36.5	34.4	35.7	35.6	33.9	34.6	3.4	0.5	1.5	3.9	2.4	2.6
After thyroidectomy........	37.4	36.6	37.0	36.1	34.2	35.2	35.1	32.5	34.1	3.3	0.5	1.8	4.9	1.5	2.9

* Twenty-four hour period during the latter part of, and immediately following, the administration of digitalis.

temperature of air within 1 mm. of the surface is less than 1.0° C. below that of the skin; and within 0.5 mm., less than 0.2° C. below. (The diameters of most thermal junctions are 0.3 mm. or less.) A more frequent source of error encountered in the use of thermocouples is due to conduction of heat away from the site of measurement by the wire of the thermal junction itself. This error can be reduced, however, to less than 0.05° C. by allowing a sufficient length of wire next the junction to come into contact with the skin or by using wire of small caliber and low conductivity. The whole error involved in making measurements by means of thermocouples of the type employed is probably not large ($\pm$ 0.5° C.) and in the present study is of little importance since relative, rather than absolute, accuracy has been sought.

The second observation concerns the choice of sites of the surface of the body for study and the significance of averaging the measurements of their temperatures; the points are not of course regarded as representing the average temperature of the whole surface. The feet and hands were given as great weight in the calculation of averages as the rest of the body because they are the regions of the body most variable in temperature and are capable of losing more heat both by radiation and evaporation than any other surface of like area. The average values of the points measured are, however, comparable from time to time since the points chosen were always the same.

It is far from surprising to find a fairly regular diurnal variation in temperature of the surface of the body. If freedom of movement and of position during periods of examination were curtailed and food withheld, many minor irregularities apparent in the present data might be eliminated. The unexpected variations encountered do not, however, obscure the regular fluctuations. Considerable interest attaches to the fact that the temperatures of the interior and of the exterior of the body (more especially the exterior of the extremities) vary in opposite directions; the rectal temperature reaches its maximum while that of the extremities is low. Then that of the extremities rises and remains elevated while the rectal temperature begins its decline. The persistent inverse relation between the two suggests at once that surface temperature regularly plays a part in regulating the extent of diurnal variation of temperature in the interior of the body. Warming certain portions of the brain (17) or the blood going to the brain (18) or warming the whole body (12) is followed uniformly by peripheral vasodilatation and lends weight to such a view. When the environment is warm enough little fluctuation in the surface temperature occurs (Fig. 2b) yet variation in rectal temperature continues. Fluctuation in the production of heat which is well known probably accounts for these changes in the rectum. To elucidate this phenomenon the variations in consumption of oxygen in one instance have been plotted (Fig. 2b).

The relation of rise of temperature of the extremities to onset of

drowsiness or sleep, so much like the rise observed to occur at the onset of anesthesia, is interesting because it is associated with change in state of many other bodily functions. Rectal temperature decreases, heat production falls off, decrease in tone of skeletal muscle occurs, speed and accuracy of performance (19) is impaired. Even in the absence of sleep diurnal variations apparently occur. One can scarcely escape the conclusion that all of these changes are dependent upon fluctuation in the state of the entire nervous system.

Even in the presence of disease the general form of diurnal variation of internal and surface temperatures remains relatively undisturbed although the level at which variations occur and the extent of variation may be considerably altered. In fever associated with diseases of different natures these alterations appear to be different in character. The inference is that elevation of internal temperature may be brought about by more than one means.

A full explanation of the greater difference in temperature between surface and interior in patients with heart failure requires complete knowledge of all the factors involved. It is probable that the phenomenon is not always dependent upon the same train of events. In certain instances a very simple mechanism may prevail. In Case X (Fig. 5) for example, the temperature of the surface of the extremities never rose during the twenty-four hours to a point where heat was lost more rapidly than it was produced. The rectal temperature did not fall, therefore, until after the administration of digitalis. In this case the sequence of events may be similar to that occurring in a water bath designed to keep a uniform temperature and maintained by a constant source of heat near the center, after the stirring mechanism is slowed or stopped. The periphery cools, the central, continuously heated portion becomes warmer until a new and steeper gradient is reached and a new thermal equilibrium is established. Something of this sort apparently occurs also in sudden death except that after a period of time the source of heat disappears. Attention was called in the previous paper (1) to Ackermann's observation (20) that the temperature of the body of dogs continued to rise after the heart stopped beating while the extremities cooled. That the rise of internal temperature is dependent on cessation of the circulation and not of the respiration was demonstrated by Heidenhain (21) as well as by Ackermann. The same phenomenon has been observed in man by Wunderlich (22).

A different situation is exhibited by Case XI (Fig. 6). The hands and feet remained cold during the day and the rectal temperature rose above normal. At a given time the temperature of the surface of the extremities suddenly rose, however, to a point quite as high as ever occurs in health, and the rectal temperature promptly returned to normal. A much more complex mechanism must here be operative for it is difficult to suppose that during the afternoon the heart is unable to deliver a sufficient volume of

THE SIGNIFICANCE OF THE VESSELS OF THE SKIN IN ESSENTIAL HYPERTENSION

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Evidence of a varied nature has led to the widely accepted conclusion that one of the significant factors leading to elevation of the blood pressure in essential hypertension is constriction of the arterioles. A thorough review of the literature dealing with this problem has recently been published by Fishberg (1) and tends to show that the most important vascular territory involved in this mechanism is the splanchnic region.

The evidence is, as has been said, varied. As long ago as 1875 Litten (2) observed that tying off the superior mesenteric artery in dogs was followed promptly by rise of arterial pressure of from twenty to twenty-five millimeters of mercury. The elevation frequently persisted for several hours. Longcope and McClintock (3, 4) demonstrated that compression of the coeliac axis and superior mesenteric arteries in dogs is followed by temporary increase of the blood pressure. Jansen, Tams and Achelis (5) repeated these experiments and essentially confirmed their results. They observed further that cold colonic irrigations in normal individuals were followed by much greater rises in blood pressure than were cold baths although in the case of colonic irrigations constriction probably involved only the colonic section of the splanchnic vessels, whereas in cold baths nearly all the vessels of the skin were affected. They found that binding the extremities of normal persons with elastic bandages caused only an insignificant rise in blood pressure but that in individuals suffering from essential hypertension it was attended by marked elevation of the arterial tension. This phenomenon was interpreted as indicating that in patients with arterial hypertension reduction in the degree of distensibility of the splanchnic vessels resulted in failure to attain normal compensatory dilatation following constriction of peripheral vessels. In extensive studies on the action of pressor substances prepared from the plasma of normal and hypertensive individuals Page (6) found likewise that it was constriction of the blood vessels of the splanchnic region that was chiefly responsible for rise in blood pressure on injection of such substances into animals. Similarly, it has long been known that the vessels of the splanchnic region are most important in the rise of blood pressure following injections of adrena-

lin. On the other hand many observations in patients with thrombo-angi-itis obliterans substantiate the conclusions of Jansen, Tams and Achelis that no relation exists between the narrowing of the arterioles of the extremities and the level of systemic blood pressure (1). It has been repeatedly demonstrated in this clinic, moreover, that attacks of spastic contractions of peripheral arteries resulting in numbness and paleness of the skin—a common occurrence in patients with essential hypertension—are not accompanied by rise in arterial pressure.

In interpreting experiments in animals it should be emphasized, however, as do Meyer and Gottlieb (7) that the reactions of the vessels of the skin in human beings may differ widely from those of the vessels of the skin in fur-bearing animals.

Although the results of previous investigations appear to indicate that the vessels of the skin do not participate in the mechanism which results in elevating the blood pressure in essential hypertension it seemed desirable to test this view with evidence of a different nature. Since the temperature of the surface of the body depends almost entirely upon the flow of blood to the skin, measurements of surface temperature were undertaken over a 30-hour period under uniform conditions to secure new information on the behavior of the arterioles in this malady.

The technique and conditions of measurement of surface temperature together with data obtained by this technique in normal individuals have been published (8). In this study the behavior of the vessels in the skin of nine individuals, three males and six females, suffering from arterial hypertension was investigated. Their ages fell between 29 and 48 years. They were selected because evidence of heart failure, of diminution in function of the kidneys as measured by the urea clearance test of Van Slyke, and of arteriosclerosis was absent. The duration of hypertension varied from eighteen months to five years. Several cases represented the type usually designated as " malignant hypertension."

It is evident (Fig. 1) that the form of the curve of surface temperature in the hypertensive group during a twenty-four-hour period is similar to that of normal individuals and that the level of the two groups is approximately the same. It follows that the average temperature of both groups for the whole period is also the same (Table I). No significant alteration in either systolic or diastolic arterial pressure was observed furthermore during the diurnal changes in surface temperature in the group as a whole or in any individual case. When the arterial pressure is high some increase in tone of the peripheral vessels must develop in order to maintain a normal flow of blood through the skin. That the latter is true is shown by the fact that the temperature of the skin is normal. If there were no increase in arterial tone in the presence of high arterial tension, increased flow of
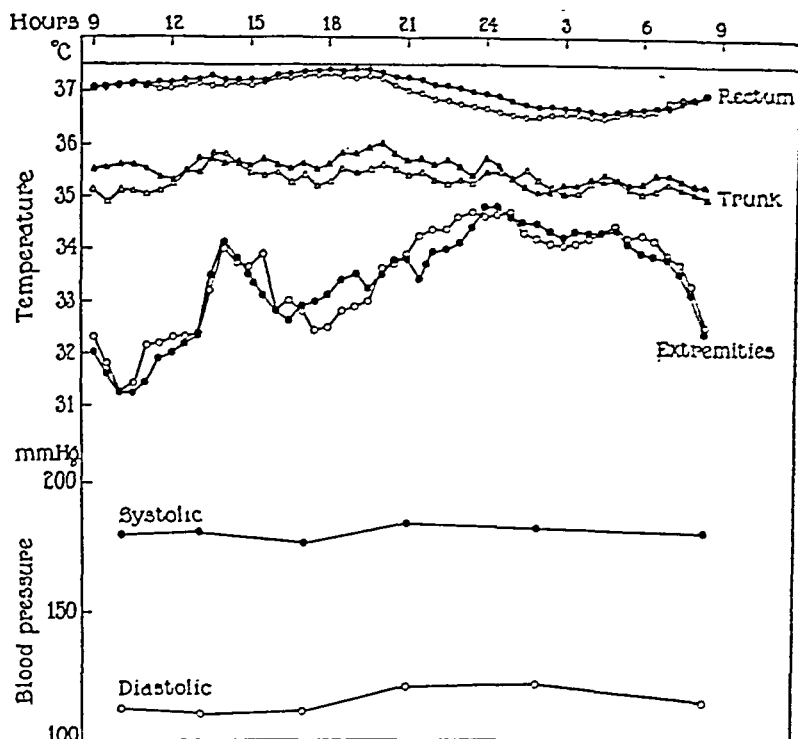
FIG. 1. FIGURES FOR THE AVERAGE TEMPERATURES OF THE DESIGNATED LOCATIONS IN THE GROUP OF NORMAL INDIVIDUALS ARE CHARTED IN SOLID SYMBOLS; THOSE FOR THE GROUP OF HYPERTENSIVE INDIVIDUALS IN OPEN ONES.

blood through the skin would result in excessive loss of heat. The slight increase in tone of the arterioles of the skin may be regarded, therefore, as dependent upon, rather than antecedent to, general arterial hypertension.

CONCLUSIONS

1. The temperature of the skin of individuals suffering from arterial hypertension does not differ significantly from that of normal individuals.

2. Diurnal variations in surface temperature regularly occur in individuals with arterial hypertension without significant change in arterial pressure.

3. Elevation of arterial pressure in hypertensive individuals does not depend on, though it may be accompanied by, constriction of the arterioles of the skin.

TABLE I

Temperatures of rectum, trunk, and extremities obtained for individual cases, and combined averages for each of the two groups

Type of patient	Rectum			Trunk			Extremities			Differences between Rectum and Trunk			Extremities		
	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.	Maximum °C.	Minimum °C.	24 hour average °C.
Normal															
Case I	37.4	36.2	36.9	36.2	34.3	35.4	34.7	31.9	33.5	3.1	0.7	1.5	5.5	2.2	3.4
II	37.4	36.5	36.9	35.6	34.2	34.8	33.9	30.9	32.5	3.2	0.9	2.1	6.5	2.6	4.4
III	37.5	36.9	37.3	36.7	35.6	35.9	35.8	32.3	34.9	1.9	0.2	1.4	5.2	1.1	2.4
IV	37.4	36.7	37.1	35.9	34.7	35.0	34.3	29.6	33.1	2.7	0.8	2.1	6.3	2.4	4.0
V	37.5	36.5	37.1	37.0	35.0	35.8	35.9	28.2	32.6	2.5	0.1	1.3	9.3	0.6	4.5
VI	37.5	36.6	37.2	36.0	34.9	35.4	34.5	31.6	33.3	2.6	0.6	1.8	5.9	2.1	3.9
Average	37.4	36.6	37.1	36.2	34.8	35.4	34.8	30.7	33.3	2.7	0.5	1.7	6.4	1.8	3.8
Hypertension															
Case I		37.3	36.5	36.8	35.4	33.8	35.0	31.5	33.5	3.5	1.1	1.8	5.8	1.4	3.3
II		37.6	36.6	37.1	36.4	34.2	35.2	29.1	32.0	3.4	0.2	1.9	8.5	1.6	5.2
III		37.6	36.3	36.9	36.2	33.0	35.2	29.5	33.0	4.6	0.1	1.7	8.1	1.0	3.9
IV		37.2	36.0	36.6	36.1	35.0	35.7	30.6	32.8	2.2	0.1	0.9	6.6	1.3	3.8
V		37.2	36.3	36.7	36.2	33.3	34.7	31.0	34.1	3.9	1.6	2.0	6.2	0.8	2.6
VI		37.5	36.5	36.9	36.1	33.5	35.1	28.4	31.8	4.0	0.4	1.8	9.1	1.1	5.1
VII		37.6	36.7	37.1	35.8	33.3	34.8	33.3	34.5	4.3	0.9	2.3	4.3	1.1	2.6
VIII		37.5	36.7	37.0	36.9	34.8	35.5	30.4	34.4	2.7	0.2	1.5	7.1	0.6	2.6
IX		37.7	36.7	37.2	36.6	34.3	35.8	31.1	33.6	3.4	0.1	1.4	6.6	1.3	3.6
Average	37.5	37.5	36.5	36.9	36.2	33.9	35.2	30.5	33.4	3.6	0.5	1.7	6.9	1.1	3.6

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THE NEUFELD METHOD OF PNEUMOCOCCUS TYPE DETERMINATION AS CARRIED OUT IN A PUBLIC HEALTH LABORATORY: A STUDY OF 760 TYPINGS [1]

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As early as 1902 Neufeld (1), in Germany, noticed that when pneumococci are mixed with homologous immune serum, there occurs a pronounced swelling (quellung) of the pneumococcus capsule. In 1931 and 1932, Armstrong (2) (3), in England, reported his results in typing pneumococci in sputum and mouse peritoneal exudate using a method based on this phenomenon: these results were confirmed by Logan and Smeall (4). Last year, Sabin (5) of the Bellevue Hospital, New York, reported his findings using the Neufeld method for the typing of pneumococci in sputa. During the past 14 months this method has been used as a routine procedure at the Bacteriological Laboratory of the Massachusetts Department of Public Health—and has been found to be simple and accurate.

The technic used during this period was demonstrated to us by Dr. Kenneth Goodner of the Hospital of the Rockefeller Institute. His technic has been modified to the extent that plane slide preparations have been used in preference to hanging drops. Furthermore, we have extended the Neufeld method, previously used only for the determination of Types I, II and III, to the determination of the other 29 specific types of pneumococci.

Our technic is as follows: upon receipt of the sputum at the laboratory, stained liquid mounts of the specimen are mixed with rabbit antisera (Types I to XXXII) used undiluted. Combinations of monovalent antisera (rabbit) are used instead of making thirty-two preparations of the sputum with the thirty-two monovalent sera. The combinations of sera that we use are the following:

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Type I
A. Types II, IV, V and VII
B.   "    III and VIII
C.   "    IX, XI, XIII and XV
D.   "    VIa, VIb, XVII and XVIII
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E, Types XII, XIV, XVI and XXVIII
F, " X, XIX, XX and XXI
G, " XXII, XXIII, XXIV and XXV
H, " XXVII, XXIX, XXX, XXXI and XXXII

Nine loopfuls of sputum are placed approximately one inch apart on a 9 x 2 inch plane glass slide; to each drop is added two loopfuls of the antiserum, i.e. the first drop is mixed with Type I antiserum, the second drop is mixed with combined serum A (II, IV, V and VII), the third drop with B, etc. These preparations are stained with Loeffler's alkaline methylene blue (two drops to each mixture) and are covered at once with cover slips to prevent drying. Examination is made with the oil immersion lens, with the light dimmed. When a positive reaction occurs, which is usually within a few minutes, there is a decided swelling of the capsule of the pneumococcus present. The swollen capsule is of a light greenish-grey color, is much less translucent than one that is not swollen, and has a very
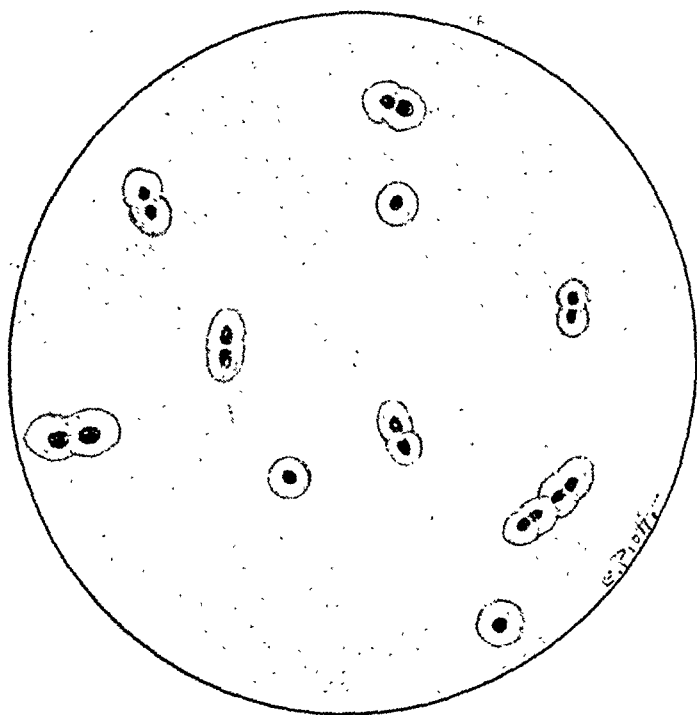
PLATE A. Positive reaction showing swollen capsules.

definite outline which is one of the most characteristic features of a positive reaction (Plate A). In the preparations in which no reaction is evident, the capsule of the pneumococcus appears as a halo of refracted light (Plate B). In all preparations the body of the pneumococcus stains a definite blue. If the reaction is observed in drop 1, then the pneumococcus present is Type I and can be reported immediately; if the reaction is ob-

served in drop 3, for example, the test is repeated using two drops of the sputum mixed with Type III and Type VIII undiluted monovalent rabbit sera, respectively; if the reaction is observed in drop 9. five loopfuls of the sputum are mixed with Types XXVII. XXIX. XXX. XXXI and XXXII undiluted monovalent sera. respectively. Should no reaction be seen on the first examination, the preparations are reexamined at the end of thirty minutes.

When dealing with sputa containing many Type III organisms. it has been necessary, occasionally, to dilute the sputum with saline before any
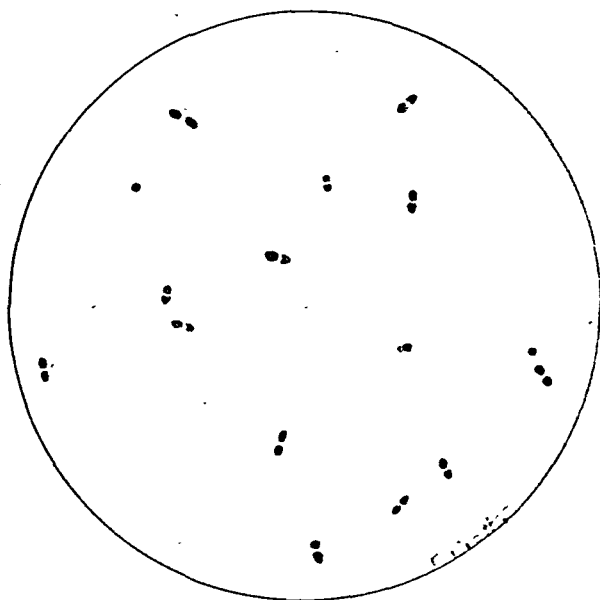
PLATE B. Negative reaction (capsules not swollen).

"quellung" of the pneumococcus capsule became evident. In such instances, when we have used the regular Neufeld technic. the organisms formed large masses which were surrounded by much precipitated material; and no definite swelling of the capsules could be seen. However. upon dilution of the sputum with saline and the test repeated. the individual diplococci have shown a typical positive reaction.

The specimens of sputum that we receive are often at least 24 hours old, since they are sent by mail from various parts of the State. In our hands. the age of the specimen has made little difference in relation to the success with which the Neufeld method has been applied. We have demonstrated a typical positive reaction on sputum over 48 hours after the time of its collection from the patient.

During the past 16 months, 760 specimens of sputum have been examined by this method; of these, 246 were diagnosed immediately as Types I, II and III, and 110 as pneumococci of the higher types.[2] Nine others were questioned, two as Type I's, three as Type III's and four as Group IV's. These later proved to be the types suspected.

TABLE I

Results of Neufeld method on 760 specimens

Number of sputa found to have specific types (I–XXXII).............. 356
     "   "   "   in which specific type was doubtful.................. 9
     "   "   "   set up for Types I, II and III only, later classified as
                 higher types................................... 148
     "   "   "   showing typical pneumococci, negative on Neufeld..... 25
     "   "   "   in which typical pneumococci were not seen, pneumo-
                 cocci cultivated later........................... 102*
     "   "   "   not containing pneumococci...................... 120
      Total... 760

* Of these, 9 showed Type I pneumococci and 4 Type II, and the remainder were those of higher types.

The results of the 356 positive Neufeld typings have been confirmed by the various methods shown in Table II.
Whenever the amount of sputum was sufficient, as many of these methods were used on the individual specimen as was possible—thus making as

TABLE II

Confirmation of Neufeld results by other methods

Type	Mouse methods only	Krumwiede only	Methods on Avery broth only	Mouse methods and Krumwiede	Mouse methods and Avery broth	Krumwiede and Avery broth	Krumwiede, mouse methods and Avery broth	Other methods*	Not confirmed by any method	Total
I..........	49	16	4	32	13	4	8	1	8	135
II..........	9	2	0	9	6	2	1	0	0	29
III..........	41	6	3	12	12	1	4	1	2	82
IV–XXXII.	67	1	3	1	27	1	0	2	8	110
Total.....	166	25	10	54	58	8	13	4	18	356

* 1 Rosenthal and Sternberg's method on sputum (9).
  3 direct microscopical agglutination on sputum.

thorough and complete a study of the accuracy and efficiency of the Neufeld method as the specimens allowed.

[2] The Group IV examination by the Neufeld method has been a routine procedure for only two months.

Of the 135 sputa diagnosed Type I by the Neufeld, the standard mouse methods [3] failed to give a Type I diagnosis in 25 instances: in 12 of these, pneumococci of other types (from Types IV to XXXII) were recovered, and in the remaining 13, the mice failed to develop a pneumococcus infection. However, an agreement with the Neufeld was obtained by other methods in 17 of these cases: 7 were diagnosed Type I by the Krumwiede method (10); 4 by various methods used on the Avery (6) broth culture of the sputum; and 6 by using the Neufeld method on the peritoneal exudate of the inoculated mice. In 4 of these 6 cases, the mice died of a pneumococcus infection other than Type I, whereas the peritoneal and heart's blood cultures from the other two mice were so mixed it was impossible to isolate any pneumococci from them. Nevertheless, occasional Type I pneumococci were seen in the peritoneal exudate using the Neufeld technic on these 6 mice.

Of the 8 specimens reported Type I by the Neufeld in which we were unable to obtain a Type I diagnosis by other methods, 4 showed other types of pneumococci in the mice, and in the remaining 4, no pneumococci of any type were isolated (Table III).

In only 1 of the 29 sputa diagnosed Type II by the Neufeld method did the inoculated mouse die of infection from pneumococci other than Type II. In this instance, both the Neufeld and Krumwiede methods gave a Type II reaction on the sputum, whereas the mouse developed a Type I

TABLE III

Results of typing 246 Type I, II and III specimens by the Neufeld method

Type	Total typed by Neufeld	Checked	Not checked	Per cent checked
I.............	135	127	8*	94
II............	29	29		100
III...........	82	80	2†	98
Total.......	246	236	10	96

```
 * 1—Type III.
   1— "   IV.
   1— "   VIII.
   1— "   XXI.
   2—No pneumococci.
   2—Mouse survived.
 † 2—Type VIII.
```

[3] The standard mouse methods used were
(1) The Sabin stained slide microscopic agglutination test (7),
(2) The tube agglutination and precipitin methods (8),
(3) Microscopic or macroscopic agglutination of the heart's blood culture

septicemia. Particular mention is made of the Type I and Type II cases because of the necessity of their early and correct diagnosis in view of the serum treatment of these patients. In 2 of the sputa diagnosed Type III by the Neufeld, Type VIII pneumococci were recovered from the mice (Table III).

Because of their scarcity, pneumococci may not be seen in some sputa on microscopic examination. Satisfactory results can frequently be obtained in such instances by inoculating an Avery broth culture with 0.5 cc. of the sputum. This is incubated for from 4 to 6 hours at 37° C. Pneumococci obtained from such cultures may then be typed by the Neufeld method.

On some occasions, where 2 types of pneumococci were present in the sputum in unequal numbers, only one type was found by the Neufeld technic. At such times pneumococci of the predominant type were readily found while those of the less common type were overlooked and only found later. In these, the second type was found by the Neufeld technic applied to pneumococci obtained from an Avery broth culture, mouse peritoneal exudate, or culture of the heart's blood of the mouse inoculated with the specimen in question. Such instances were uncommon and occurred only a few times among the 760 specimens examined. Conversely, however, as noted above, pneumococci of Types I or II were in some instances found by the Neufeld method that would have been missed by other technics, owing to the heavy contamination of the specimen with other organisms.

By use of the Neufeld typing method we have been able to give physicians an immediate report on 94.6 per cent of our Type I, II and III specimens in which pneumococci were seen in the sputum, and in 85.2 per cent of all Type I, II and III specimens on which this method was tried. Particular interest is attached to the rapid determination of Types I and II because of the availability of therapeutic serum; in 89 per cent of all specimens containing these types, immediate type diagnosis was made possible by this method.

SUMMARY

The Neufeld method of pneumococcus type determination has been used for the diagnosis of Type I, II and III pneumococci for 16 months. The results obtained have been confirmed by other methods in 96 per cent of the instances.

The use of this method of typing has enabled us to give to physicians an immediate type diagnosis on 94.6 per cent of specimens containing pneumococci of Types I, II or III, when typical pneumococci were seen in the sputum.

During the past 2 months the use of the method has been extended to include the determination of the higher types (Types IV to XXXII).

This method of typing may be carried out on a very small amount of sputum, is simple, rapid and accurate, and does not require the use of mice.

CONCLUSIONS

We believe the Neufeld method of typing to be very valuable. It has enabled us to give immediate reports to physicians in 89 per cent of Type I and II cases, thus leading to earlier and consequently more effective serum therapy.

The use of the Neufeld technic for typing pneumococci contained in sputum and Avery broth cultures of it has proved an efficient routine procedure for pneumococcus type determination.

This method of typing has proved as accurate as other more generally used methods. Its outstanding advantage is that a type diagnosis can be made almost immediately (5 to 30 minutes).

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THE EFFECT ON RENAL EFFICIENCY OF LOWERING ARTERIAL BLOOD PRESSURE IN CASES OF ESSENTIAL HYPERTENSION AND NEPHRITIS

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In 1856 L. Traube (1) postulated that arterial pressure was elevated in cases of disease of the kidneys to overcome mechanical resistance against blood flow, thus compensating for the abnormal resistance and maintaining the efficiency of the kidneys as excretory organs. The " compensatory " theory has had many adherents, some of whom have generalized it to include hypertension of varied etiology.

The object of the present investigation was to compare the efficiency of excretion, when the blood pressure was at a high level, with that when it was reduced. It was hoped to bring evidence which would substantiate or refute the compensatory theory as applied to patients suffering from hypertension. The urea clearance test of Möller, McIntosh and Van Slyke (2) was used for the comparison.

METHOD

Two patients exhibiting extreme elevation of blood pressure and typical histories of the malignant phase of essential hypertension, two with moderate hypertension, and two suffering from hemorrhagic Bright's disease, were selected for this study. Throughout the control period of two months or more, and the experimental period of from three months to a year, blood pressures were taken at 9:30 A.M. with the patient confined to bed at all times. Control urea clearance tests were performed. The arterial blood pressure fell spontaneously in two cases (Numbers 1 and 6) sufficiently to be significant. Two patients (Numbers 3 and 4) received sodium thiocyanate by mouth in doses graduated from 65 mgm. to 260 mgm. given daily until the systolic blood pressure had fallen 80 mm. Hg or more (3, 4, 5, 6). Clearance tests were again run and the blood pressure again allowed to approach the original level by discontinuance of thiocyanate. One patient (Number 2) exhibited persistently elevated blood pressure for a period of at least five years. Intramuscular injection of aqueous colloidal sulfur (1 cc. 1 : 1000 solution) was associated with a sharp and prolonged fall in pressure to normal (7). The blood pressure

TABLE I

Effect of alteration of arterial blood pressure on renal efficiency as measured by the urea clearance test

Patient number	Date	Blood pressure	Corrected urine volume	Blood urea	Urea clearance		Treatment	Diagnosis
		mm. Hg	*cc. per minute*	*mgm. per cent*	*Successive hourly periods per cent of normal*	*Average per cent of normal*		
1	April 3	210/120	1.2 1.0	15.7	58.2 69.6	63.9	None	Essential hypertension
	October 10	118/116	4.5 4.1	6.4	70.2 67.1	68.6		
	October 23	150/100	4.5 4.1	6.9	68.0 70.4	69.2		
	November 9	190/120	4.2 4.0	8.8	62.2 59.6	60.9		
2	April 17 (1933)	204/110	.32 .73	19.6	16.6 24.9	20.7	None	Essential hypertension
	May 1 (1934)	230/124	1.9 2.3	24.7	38.3 41.3	39.8		
	May 7	220/120	1.3 2.2	21.9	29.2 29.7	29.4		
	June 8	138/92	2.3 3.4	16.5	36.3 30.4	33.4	Sulfur injection 1 c.c. 1 : 1000 Q.D.	
	June 11	128/88	3.9 2.7	19.4	42.1 34.6	38.3		
3	June 1	264/118	1.4 0.8	14.0	47.5 49.3	48.4	None	Malignant hypertension

TABLE I (continued)

Patient number	Date	Blood pressure	Corrected urine volume	Blood urea	Urea clearance		Treatment	Diagnosis
					Successive hourly periods per cent of normal	Average per cent of normal		
		mm. Hg	cc. per minute	mgm. per cent				
3	June 13	228/108	0.9 / 0.9	19.8	51.9 / 49.7	50.8		
	December 6	278/118	2.0 / 5.0	15.8	44.0 / 44.4	44.2		
	January 16	238/136	0.8 / 3.4	12.2	55.0 / 43.8	49.4	Thiocyanate	
	February 25	220/108	0.5 / 3.2 / 2.9	14.2	64.8 / 43.0 / 74.2	60.6	Thiocyanate	
	March 6	266/130	0.4 / 0.6	13.0	54.2 / 51.2	52.7		
	March 18	290/137	0.4 / 2.5	17.9	47.8 / 37.0	42.4		
	March 20	274/130	2.8 / 0.4	9.3	46.9 / 73.7	60.3		
	April 8	230/130	2.2 / 0.6	12.8	50.4 / 47.8	49.1	Thiocyanate	
4	January 25	300/163	1.0 / 3.4	15.4	53.5 / 58.0	55.7	None	Malignant hypertension

TABLE I (continued)

Patient number	Date	Blood pressure	Corrected urine volume	Blood urea	Urea clearance Successive hourly periods	Urea clearance Average	Treatment	Diagnosis
		mm. Hg	cc. per minute	mgm. per cent	per cent of normal	per cent of normal		
4	January 31	250/154	2.0 4.4 7.4 5.3	13.2 11.8	50.0 62.6 52.4 63.4	59.6		
	February 8	240/150	1.8 2.3 9.2 8.5 4.4	11.8 11.8	67.0 63.6 73.6 69.3 63.2	65.3		
	February 25	220/132	2.0 6.4	11.2	71.6 84.2	77.9	Thiocyanate	
	March 18	200/120	2.0 5.0	16.7	61.1 76.8	68.9	Thiocyanate	
	April 3	220/122	1.8 3.9	10.8	71.2 80.3	75.7	None	
5	February 15 (1933)	188/104	1.0 1.7 4.3 4.3	14.5 17.0	50.6 52.2 42.2 42.5	46.4	None	Chronic nephritis
	March 9	210/106	3.2 1.2	24.8	50.0 55.0	52.5		
	November 2	160/102	1.8 3.9	16.5	45.0 53.7	49.3		
	February 10 (1934)	154/86	1.9 3.1	15.7	46.9 54.1	50.5		

TABLE I (*continued*)

Patient number	Date	Blood pressure	Corrected urine volume	Blood urea	Urea clearance		Treatment	Diagnosis
					Successive hourly periods per cent of normal	Average per cent of normal		
		mm. Hg	cc. per minute	mgm. per cent				
5	February 23	150/90	1.9 2.3	18.2	47.7 47.3	47.5		
	March 5	160/98	.9 .7	32.2	64.2 56.4	60.3		
	March 23	136/80	1.2 1.9	14.8	59.9 42.8	51.4	Denervated right kidney	
	March 27	132/80	1.3 1.9	12.9	75.9 48.4	62.1		
6	March 11	194/110	0.45 1.0	46.3	13.5 14.9	14.2	None	Terminal stage of hemorrhagic Bright's disease
	March 21	174/100	0.67 0.90	61.3	12.6 13.0	12.8		
	April 4	160/100	0.68 1.0	113.0	9.4 10.5	9.9		
	April 7	116/82	1.2 1.0	114.0	9.5 10.2	9.3		
	September 27	138/86	1.6 1.8	105.0	11.3 11.9	11.6		
	October 28	122/78	1.1 1.0	147.3	9.8 9.6	9.0		
	March 1	154/94	0.67 1.0	43.3	11.7 8.0	9.8		
	April 2	168/102	1.1 1.5	130.0	7.7 8.2	7.9		

in one patient (Number 5) fell after denervation of one kidney was performed. The results are presented in the following table.

The blood pressure figures represent the blood pressure at the time the clearance was performed, also the approximate level for days or weeks previous to or following the test.

DISCUSSION

Whether reduction in blood pressure occurred spontaneously or resulted from sodium thiocyanate, colloidal sulfur injections, or unilateral renal denervation, no significant change in the clearance resulted. Nor did the clearance change when the pressure returned to its original level. It must be concluded that sodium thiocyanate and sulfur in the dosage employed and unilateral renal denervation had no detrimental effect on renal function.

Reid (8) found that administration of nitrites in therapeutic doses did not diminish the ability of the kidneys to concentrate urea in the urine, after its administration. The diuresis which ordinarily follows administration of 15 grams of urea is usually reduced by drugs of the nitrite series. Large doses of nitrite cause intolerance to the drug long before the stage of suppression of urine excretion.

CONCLUSIONS

1. The efficiency of the kidneys, as measured by the urea clearance test, is not altered by a marked fall in arterial blood pressure occurring spontaneously, or induced by sodium thiocyanate administered by mouth, or colloidal sulfur administered intramuscularly, in patients suffering from essential hypertension.

2. Sodium thiocyanate or colloidal sulfur in the dosage employed and over short periods of time does not appear to have a detrimental action on the kidneys of patients suffering from essential hypertension.

3. Fall in arterial blood pressure occurring spontaneously or as the result of renal denervation in patients suffering from chronic Bright's disease also caused no change in renal efficiency.

4. The abnormal elevation of blood pressure in these cases does not appear to assist in maintenance of renal efficiency. This evidence does not support the compensatory theory of the cause of hypertension in patients suffering from nephritis or essential hypertension.

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THE ACIDOSIS OF GUANIDINE INTOXICATION [1]

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Recent reports (1) (2) (3) (4) (5) which implicate guanidine intoxication as a complicating factor in some instances of acute liver injury, of eclampsia, and of alimentary intoxication in infants, make it desirable to know in greater detail the effect of guanidine on various biological processes. Only in this way can we know in what manner increased guanidine may modify the clinical picture in a given disease and may influence the response of the patient to treatment of his symptoms. Since acidosis must often be treated in the presence of hyperguanidinemia, it is of interest to study the effect of guanidine intoxication on the acid base equilibrium.

Various observers have mentioned breathing of the " air hunger " type in guanidine poisoning. Watanabe (6) reported a decrease in pH and CO_2 of the blood and an increased output of ammonia in the urine of experimental animals poisoned with guanidine. His results were disputed by György and Vollmer (7) who argued that a condition of alkalosis rather than acidosis must be produced since the hyperirritability caused by guanidine can be relieved by the administration of acid. These authors, however, presented no studies of acid base equilibrium to substantiate their claims. We already have considerable evidence that one feature of the intoxication is a serious disturbance of lactic acid metabolism. In an investigation of the hypoglycemia induced by guanidine and by its derivative synthalin, Staub (8) showed that lactic acid accumulates in the tissues and body fluids and is excreted in the urine. He attributes this accumulation to the inability of tissues poisoned with guanidine to oxidize and resynthesize lactic acid to its precursors in a normal manner. Gradually more and more of the carbohydrate reserve is transformed to lactic acid until a severe hypoglycemia results. Minot (9) confirmed these observations both in experimental guanidine poisoning and in the intoxication which occurs when hyperguanidinemia is a result of acute extensive necrosis of the liver. Although no acid base studies were reported in connection with either of these investigations one would expect that, as lactic acid accumulates and is neutralized, there would be a progressive depletion of the alkali reserve.

In order to study the effect of guanidine on the acid base balance of the subject or on his response to medication, it is necessary to use animals with experimental poisoning. Here one can observe the intoxication uncomplicated by disease and can study the response to a single form of medication.

EXPERIMENTAL

Intoxication was produced in dogs by the subcutaneous injection of guanidine hydrochloride. Because of the marked influence which the state of calcium nutrition has on susceptibility to guanidine poisoning, the dogs were kept on uniform diets for three or four weeks before the experiments were started. The diet of raw lean beef without bones was low in calcium. As a rule the dogs showed typical severe intoxication following the administration of two or three daily doses of 100 mgm. of guanidine hydrochloride per kilo of body weight. Three or four times as many such doses are required to produce the same severity of intoxication in dogs on a high calcium intake. Determinations of pH, CO_2 content, lactic acid, sugar, nonprotein nitrogen and guanidine were carried out on samples of blood as the intoxication progressed. The determinations were made by the following methods: pH by the colorimetric technique described by Cullen (10), CO_2 by the manometric method of Van Slyke and Neill (11), lactic acid by the method of Friedemann and Kendall (12), sugar by Benedict's method (13), nonprotein nitrogen according to Folin and Wu (14), and guanidine by the colorimetric technique of Major and Weber (15), as modified by Minot and Dodd (1).

A marked fall in the pH and alkali reserve of the blood was invariably found to occur when the intoxication became severe. Traces of acetone were occasionally found in the urine and in some instances a retention of nitrogen indicated a nephritis which may have contributed to the acidosis. The most consistent finding, however, was a marked increase in the lactic acid content of blood and urine. We apparently had an acidosis due mainly to the replacement of bicarbonate of the body fluids by lactate. The highest concentrations of lactate in the blood were found during periods in which fibrillary tremors and convulsive twitching greatly increased the muscular activity. Although increased production was in these instances an obvious cause for the accumulation of lactic acid, defective metabolism appeared also to be a factor as there was but little tendency for the lactic acid in the blood to return to a normal level even after the subject had remained quiet for a long time. Sometimes there was depression rather than hyperirritability throughout the period of intoxication but still there was a marked increase in lactic acid. These experiments emphasize further the point already made by Staub (8) and by Minot (9) that lactic acid is not metabolized normally in guanidine intoxication.

In Table I data are presented showing the increase in lactic acid in the

Development of acidosis in dogs experimentally poisoned with guanidine hydrochloride

TABLE

Dog number	Guanidine hydrochloride administration (mgm. per kilo)	Date	Sample — Time	pH	Total CO₂ (volumes per cent)	BHCO₃ (volumes per cent)	Lactic acid (mgm. per 100 cc.)	Non-protein nitrogen (mgm. per 100 cc.)	Guanidine (mgm. per 100 cc.)	Remarks
1	100 after sample; 100 at 10:00 a.m.	October 30, 1933	Preliminary 9:30 a.m.	7.35	51.6	48.9	21.2		0.48	Dog looks quite well; no definite symptoms; no acetone in urine
		October 31, 1933	2:15 p.m.	7.21	35.8	33.4	61.4	42.0	3.30	Dog comatose; jerky; convulsions when aroused;
		October 31, 1933		6.97	14.2	12.5	166.3			
2	75 after sample; 100 "; 100 at 10:00 a.m.	November 1, 1933	Preliminary	7.37	54.2	51.4	18.9	31.0	0.38	Dog perfectly well
		November 2, 1933	9:00 a.m.	7.41	52.2	49.8	18.4	26.0	1.34	Dog rather quiet but not very sick
		November 3, 1933		7.38	53.0	50.4	27.0	36.0	0.98	
		November 3, 1933	4:15 p.m.	7.10	28.6	26.0	92.5	60.0	2.20	Dog very quiet; hard to arouse; only nervous symptom is depression
3	100 after sample; 100 "	November 1, 1933	Preliminary	7.37	53.5	50.8	15.5	33.0	0.39	Dog looks well
		November 2, 1933	9:00 a.m.	7.39	45.0	42.8	35.3	30.0	1.62	Marked tremor; breathing rapidly and deeply
		November 2, 1933	5:00 p.m.	7.07	23.2	20.9	99.0	50.0	2.85	Dog very quiet; no twitching
			7:00 p.m.	7.08	26.8	24.3	74.7		2.50	Dog better than last night; walks around; hyperirritable when startled
		November 3, 1933	9:00 a.m.	7.26	34.3	32.1	65.0	48.0		

TABLE I (*continued*)

Dog number	Guanidine hydrochloride administration (mgm. per kilo)	Sample Date	Sample Time	pH	Total CO₂ (volumes per cent)	BHCO₃ (volumes per cent)	Lactic acid (mgm. per 100 cc.)	Non-protein nitrogen (mgm. per 100 cc.)	Guanidine (mgm. per 100 cc.)	Remarks
4	75 after sample	November 6, 1933	Preliminary	7.37	57.2	54.3	22.5	37.0	0.42	Dog vomited part of food in morning
	35 " "	November 7, 1933	9:00 a.m.	7.28	48.0	45.0	62.2	63.0	3.30	Dog quiet; not very sick
			3:30 p.m.	6.95	6.4	5.6	200.8	111.0	4.90	Dog unconscious; convulsions; no acetone in urine; died soon after
5	100 after sample	November 6, 1933	Preliminary	7.43	51.4	29.1	31.1	36.0	0.38	Dog looks sick; refused to eat; fine tremor
			4:30 p.m.	7.24	32.7	30.5	62.2			
	75 9:00 a.m.	November 7, 1933	4:00 p.m.	7.23	28.2	26.3	87.6	84.0	4.10	Vomited most of day; fine tremor; lies quietly in cage
6	75 after sample	November 8, 1933	Preliminary	7.36	57.6	54.6	25.1	33.0	0.40	Fibrillary twitching; frequent vomiting; lies quietly when undisturbed
	75 in a.m.	November 9, 1933	7:15 p.m.	7.07	34.6	31.2	106.0	81.0	4.60	

blood and the degree of acidosis present in acute experimental guanidine poisoning. Although a large group of animals have been studied data from only six typical experiments are included as further figures of the same kind appear incidentally in other tables in this paper. It is apparent from the figures presented that there is a tendency for the pH of the blood to fall considerably while the alkali reserve is still not greatly reduced. This tendency has been observed repeatedly throughout our study of the acidosis of guanidine poisoning. The fall in pH may be associated with a depression of the respiratory center, since some subjects with severe acidosis fail to show increased respiration of the "air hunger" type. Circulatory disturbances which are now being investigated may also play a considerable rôle in allowing free CO_2 to accumulate in the blood.

Hartmann and Senn (16) (17) (18) have recently reported that the utilization of sodium r-lactate administered intravenously furnishes base to increase the alkali reserve in normal and acidotic subjects. If the acidosis of guanidine poisoning is due primarily to an inability to metabolize lactic acid of endogenous origin there should also be a poor utilization of administered lactate. Experiments were planned to compare the effectiveness of sodium lactate with that of other therapeutic measures in the treatment of the acidosis produced in animals by the administration of guanidine.

It seemed necessary before we could give much weight to observations in regard to the utilization of lactate by dogs poisoned with guanidine to study the effect of similar doses of lactate on normal dogs and on dogs with other types of acidosis. The dose of molar solution of sodium lactate administered in each case was calculated, according to the formula given by Hartmann and Senn (16), to raise the alkali reserve of the body fluids by 20 volumes per cent. The solution was diluted with 2 to 3 volumes of water and injected into a leg vein. In a few instances when death from hypoglycemia seemed imminent 5 per cent glucose solution was used as a diluent. Blood for analysis was drawn with as little stasis as possible from the external jugular vein before and at hourly intervals after the administration of lactate. The bladder was emptied before the lactate was given and the catheter kept in place so that samples of urine could be obtained corresponding to the blood samples which were taken. The bicarbonate and lactate content of the urine was determined. Only the data showing the effect on the blood chemistry and the amount of lactate excreted unchanged in the urine are included in Table II. Typical experiments from each group of studies are presented.

Most of the increase in alkali reserve of the blood occurred during the first hour after the lactate was injected but a slight additional rise was sometimes noted in the second or third hour. The lactate in the blood had usually returned to approximately the normal level by the end of three hours. In ten normal dogs the increase in alkali reserve in the blood in

GUANIDINE INTOXICATION

TABLE II

The administration of sodium lactate to dogs with acidosis due to various causes

Dog number	Cause of acidosis	Preliminary blood chemistry				1 hour			2 hours		3 hours		Maximum change in BHCO₃ of blood	Time of maximum change	Per cent of dose excreted as lactate	Remarks
		pH	Total CO₂	BHCO₃	Lactic acid	Guanidine	BHCO₃	Lactic acid	BHCO₃	Lactic acid	BHCO₃	Lactic acid				
			volumes per cent	volumes per cent	mgm. per 100 cc.	mgm. per 100 cc.	volumes per cent	mgm. per 100 cc.	volumes per cent	mgm. per 100 cc.	volumes per cent	mgm. per 100 cc.	volumes per cent	hours		
1	Normal	7.43	56.4	53.9	19	0.37	68.9	55	71.1	38	67.0	31	+17.2	2	13.4	
2	Normal	7.36	62.5	59.3	23	0.40	74.6	62	65.9	46	71.3	27	+15.3	1	13.5	
3	Normal	7.37	55.2	52.2	17	0.38	67.6	58	67.4	51	60.5	38	+17.3	3	12.0	
4	Phloridzin diabetes	7.12	23.4	21.4	25	0.40	40.1	59	44.0	44	42.8	34	+22.6	2		Urine lost
5	Phloridzin diabetes	7.23	37.6	35.0	21	0.37	51.5	37	56.1	29	56.9	15	+21.9	3	9.0	Much acetone and sugar in urine
6	Diabetes; depancreatized dog	7.31	37.4	35.2	21	0.37	52.0	40	52.1	31	50.7	20	+16.8	1	15.1	Much acetone and sugar in urine
7	Uranium nephritis	7.17	28.2	26.0	21	0.37	42.5	53	44.7	32	37.0	20	+18.7	2	0.0	Dog anuric
8	Uranium nephritis	7.19	33.2	29.7	13	0.45	49.9		55.5		52.8	16	+25.8	2	10.0	No acetone in urine
9	Severe guanidine poisoning	7.23	38.1	35.2	88	1.70	27.0	163	33.9	144			−12.4	2	41.0	Some reaction to dose of lactate. Required artificial respiration for few minutes. Marked tremor. Experiment had to be discontinued at 2 hours
10	Severe guanidine poisoning	7.21	33.5	31.0	129	35.0	23.1	400	31.2	351	418		+ 4.0	1	24.1	Vomited and had marked fibrillary tremors following dose. Sample 4 hours after dose: BHCO₃ 25 volumes per cent; lactic acid 520 mgm.

TABLE II (continued)

Dog number	Cause of acidosis	Preliminary blood chemistry					1 hour		2 hours		3 hours		Maximum change in BHCO₃ of blood	Time of maximum change	Per cent of dose excreted as lactate	Remarks
		pH	Total CO₂ vol. per cent	BHCO₃ vol. umes per cent	Lactic acid mgm. per 100 cc.	Guani- dine mgm. per 100 cc.	BHCO₃ vol. umes per cent	Lactic acid mgm. per 100 cc.	BHCO₃ vol. umes per cent	Lactic acid mgm. per 100 cc.	BHCO₃ vol. umes per cent	Lactic acid mgm. per 100 cc.	volumes per cent	hours		
11	Severe guanidine poisoning	7.15	41.4	38.7	80	3.20	20.8	101					− 8.0	1	3.3	Looked much worse after dose. General convulsion 30 minutes after dose. Died 14 hours after dose. Hypoglycaemia. Practically anuric.
		0.05	13.4	211	5.44	11.1	315	17.4	201							
12	Very severe guani- dine poisoning	7.21	40.4	43.1								113	+5.5	+5.7	2	18.3	Dog died 30 minutes after 2-hour sample. Very restless throughout experi- ment but had no real con- vulsions. Lactate given with 5 per cent glucose. pH not improved.
					2.01	40.7	120	50.0	61	48.0						
13	Fairly severe guani- dine poisoning	7.20	43.3	33	2.34	49.0	83	38.1	61	40.8	57	+4.8	3	3		Hyperexcitable. Consid- erable muscle twitching. Could not catheterize, no urine obtained.
14	Moderate guanidine poisoning	7.10	40.0	40	40.1		80	11.1	78	41.2	50	+12.3	3	8.0		Sample taken 8 hours after dose showed increase of 12.1 volumes per cent over preliminary.
15	Moderate guanidine poisoning	20.0	31.1	1.50						17.1	13	+11.0	3	21.0		Dog quiet throughout ex- periment.
16	Very moderate gua- nidine poisoning	7.23	32.3	31.7	51	1.20	40.5	80							7.0		Dog not very sick. Showed muscular twitch- ing for few minutes follow- ing dose.

no case reached the calculated 20 volumes per cent. From ten to twenty per cent of the dose of lactate was excreted unchanged in the urine. The small discrepancy between the observed rise in $BHCO_3$ and that to be expected from the utilized lactate can be accounted for by bicarbonate excreted in the urine when the already normal level of $BHCO_3$ in the blood was raised above the renal threshold for bicarbonate. When all these factors are taken into consideration one can account for the disposal of the administered lactate with surprising exactness, considering the roughness of the approximations involved in the calculations.

Ketone body acidosis was produced in ten dogs by the administration of phloridzin and in one by complete removal of the pancreas. The phloridzinized animals usually showed slightly more than the calculated rise in alkali reserve following the administration of sodium lactate. The over-correction was probably due to the fact that as the lactate was utilized, glucose became available and base bound with ketone bodies as well as that introduced as lactate served to increase the alkali reserve. The over-correction became much more marked if the lactate was given with glucose solution. When lactate was administered to the dog with ketone body acidosis following complete pancreatectomy the overcorrection did not occur.

Acidosis secondary to severe nephritis was produced in 8 dogs by the subcutaneous injection of uranium nitrate as described by MacNider (19). A dose of sodium lactate calculated to increase the alkali reserve 20 volumes per cent was then administered intravenously as in the experiments already described. The data show a prompt utilization of injected lactate and a corresponding increase in the alkali reserve. In some instances there was a markedly increased urinary flow following the intravenous injection of fluid in subjects which had been practically anuric. The improved kidney function apparently resulted in the excretion of considerable acid so that the increase in alkali reserve was slightly greater than the expected rise.

The preliminary experiments are in accord with the reports of Hartmann and Senn. Approximately the calculated increase in $BHCO_3$ of the blood results from the intravenous administration of sodium lactate to normal dogs and to dogs with nephritic or ketone body acidosis.

The same doses of sodium lactate were much less effective in raising the alkali reserve in animals with guanidine poisoning. In subjects with a moderate degree of intoxication the utilization of lactate was slow and incomplete. In severe intoxication there was only a slight rise or at times a fall in the alkali reserve of the blood. Unless there was oliguria due to nephritis or to previous dehydration, a considerable portion of the dose was excreted unchanged in the urine. The lactic acid content of the blood remained high. The rather abrupt fall in alkali reserve observed in some instances was accompanied by an increase in the objective symptoms of

guanidine intoxication. Severe twitching of the muscles or occasionally generalized convulsions appeared following the injection of lactate. The muscular activity increased the production of lactic acid which was poorly metabolized and further reduced the alkali reserve. Even when no convulsive symptoms were produced there was often a general exacerbation of the intoxication and little or no change in the alkali reserve. The probable reason for this deleterious effect of lactate will be discussed later in this paper. The experiments just described support the hypothesis that the acidosis of guanidine poisoning is primarily due to an inability to metabolize lactic acid of endogenous origin and show the futility of attempting to correct this acidosis by the administration of base in the form of sodium lactate.

Obviously sodium bicarbonate would be directly available and, if it could be administered without untoward reactions, would be a more effective form of medication than sodium lactate. A considerable number of preliminary experiments with normal animals and with subjects with acidosis secondary to accumulation of ketone bodies and to nephritis showed that sodium bicarbonate in doses sufficient to raise the alkali reserve of the body fluids 20 volumes per cent (20) could be administered intravenously without the production of untoward symptoms. This was true even when the dose raised the base bicarbonate of the blood well above normal limits. The animals were able to make adjustments so that only a temporary compensated alkalosis was produced. In an occasional nephritic subject with anuria uncompensated alkalosis accompanied by more or less severe tetany was sometimes observed.

It soon became apparent that the administration of sodium bicarbonate to animals with severe guanidine intoxication was attended by considerable danger. The injection of a solution of this salt regularly produced an exacerbation of the symptoms of intoxication greater than that which had been observed following the administration of sodium lactate. There was often twitching of the muscles throughout the observation period and frequently there were generalized convulsions. The production of lactic acid by increased activity was so great and the disposal of lactate so defective that the alkali reserve in the blood was often further decreased an hour or two after the administration of sodium bicarbonate. Other characteristic manifestations of guanidine intoxication such as vomiting, diarrhea, and a tendency to hypoglycemia were also aggravated by bicarbonate medication. In less severe intoxication the symptoms following the injection were of the same nature but were transient and not alarming. Only in subjects with so mild a degree of intoxication that alkali therapy was hardly necessary could sodium bicarbonate solution be injected without the production of untoward symptoms. In these instances the base bicarbonate of the blood was increased by about the calculated amount.

Excess alkalinity of the blood as a cause of the undesirable reaction was

Experiments were then carried out in which calcium therapy was combined with the administration of sodium bicarbonate. Typical results obtained in these experiments are included in Table III. When calcium medication is adequate the undesirable reaction following the administration of sodium bicarbonate does not occur, and practically the calculated increase in the alkali reserve of the blood is produced. It is difficult to state exactly the amounts of calcium which constitute adequate therapy. The severity of the intoxication, the state of calcium nutrition, and the size of the subject all influence the dose required. Our usual procedure with experimental animals was to give 10 cc. of a ten per cent solution of calcium gluconate [2] before the administration of the bicarbonate and repeated smaller doses (usually 5 cc.) of the same solution whenever the reappearance of tremor indicated that more was necessary. When the hyperirritability is not adequately controlled by calcium, as was the case in Subject 5 in Table III, there is incomplete correction of the acid base equilibrium by the sodium bicarbonate. Even when calcium is administered one must guard against hypoglycemia which is so constant a feature of severe guanidine intoxication. For this reason we have found it advantageous to administer the sodium bicarbonate dissolved in a 5 per cent glucose solution.

It was noted that a considerable decrease in the degree of hyperguanidinemia resulted whenever medication involved the intravenous injection of considerable amounts of fluid. This decrease was associated with a marked increase in the output of urine. Glucose or sodium chloride solution was found as effective as alkalinizing salts in hastening the excretion of guanidine. A more detailed study in regard to the importance of fluid administration is in progress and will be reported later. In this paper we wish merely to point out that fluid administration to improve kidney function is an important feature whatever the specific means employed to combat the acidosis.

DISCUSSION

In the experiments just described the entire toxic picture was produced in otherwise normal animals by the administration of guanidine. The intoxication in these instances is therefore more severe than that which ordinarily develops as a complication in disease. An acidosis due primarily to guanidine is probably seldom, if ever, seen clinically. Similarly a less serious interference with treatment will as a rule be caused by amounts of guanidine which accumulate spontaneously. These results, nevertheless, indicate that whenever guanidine intoxication is a secondary factor in clinical disease it will to some extent favor the production of acidosis and complicate the treatment of that condition. Hyperguanidinemia is often

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[2] The calcium used was furnished by the Sandoz Chemical Works, Inc.

TABLE III

Administration of sodium bicarbonate alone and combined with calcium medication to dogs with guanidine intoxication

Dog number	Degree of Intoxication	Medication	Preliminary blood chemistry					1 hour		2 hours		3 hours		Maximum change in BHCO₃	Remarks
			pH	Total CO₂	BHCO₃	Lactic acid	Guanidine	BHCO₃	Lactic acid	BHCO₃	Lactic acid	BHCO₃	Lactic acid		
				volumes per cent	volumes per cent	mgm. per 100 cc.	mgm. per 100 cc.	vol. umes per cent	mgm. per 100 cc.	vol. umes per cent	mgm. per 100 cc.	vol. umes per cent	mgm. per 100 cc.	volumes per cent	
1	Very severe	NaHCO₃ in 5 percent glucose	6.98	10.7	17.4	200	5.40	17.0*	430	30.3	130			+ 0.5	Dog quiet and semi-conscious before dose. Violent generalized convulsions following injection. Died about 45 minutes after dose.
2	Fairly severe	NaHCO₃ in water	7.10	23.1	21.0	70	3.00	33.7	127					+12.7	Extreme restlessness and severe muscle twitchings after dose.
3	Moderate	NaHCO₃ in water	7.21	35.8	33.4	55	1.14	53.0	62					+20.5	Showed tremor and hyper-irritability after dose. No severe reaction. 6 hours later pH again had fallen to 7.28, BHCO₃ 42.5 volumes per cent; lactic acid 48 mgm.
4	Moderate	NaHCO₃ in glucose	7.10	42.7	30.3	52	3.30	50.5	00	53.4	80	47.0	00	+17.2	Marked tremor following injection, which disappeared in about an hour. Following morning pH 7.10, BHCO₃ 31.6 volumes per cent.
5	Very severe	NaHCO₃ in glucose and repeated Ca medication	7.19	31.2	31.0	65	2.50	48.0	62	12.0	65	41.5	78	+13.0	Dog semi-conscious and showed marked twitching before dose. This was relieved by Ca. No immediate reaction to dose but 1 hour later had general convulsion. Relieved temporarily by Ca. Blood sugar 30 mgm. at end of experiment in spite of glucose and Ca.
6	Severe	NaHCO₃ in glucose and repeated Ca medication	7.04	28.5	25.0	71	2.17	45.0	104	43.8	102			+20.0	Very drowsy before dose. "Air hunger." No reaction to dose. Showed only very slight tremor throughout experiment.
7	Severe	NaHCO₃ in water and calcium medication	7.25	24.7	23.1	143	1.70	46.1	201					+23.0	Dog had had convulsions before dose. Controlled with Ca and NaHCO₃. Injections; convulsion at time of injection controlled by Ca, then quiet. Died with hypoglycemia soon after first sample.
8	Moderate	NaHCO₃ in glucose and repeated Ca medication	7.23	30.3	30.0	52	1.41	57.7	74	55.0	69	55.0	01	+21.1	No reaction to dose. No tremor.

* Sample taken 35 minutes after injection of bicarbonate.

present in alimentary intoxication but certainly in this condition guanidine is at most but a secondary factor in the production of acidosis. The partial failure of subjects with alimentary intoxication to utilize lactates as reported by Hartmann and Senn (18) is probably due, at least in part, to an interference with metabolism due to guanidine. Quite possibly some of the undesirable reactions which have been ascribed to hyperalkalinity following the administration of sodium bicarbonate have in reality been due to a temporary increase in the toxic action of guanidine. Our experimental work with animals suggests that the addition of calcium therapy would make the administration of alkalinizing salts to patients with hyperguanidinemia safer and more effective. The removal of guanidine through increased kidney function may be an important feature in the adjustment of acid base equilibrium by continuous venoclysis with glucose or Ringer solution as advocated by Karelitz (25). We believe a combination of therapy consisting of forced parenteral fluids, calcium medication, and the administration of sodium bicarbonate is more satisfactory than any single procedure in the management of severe clinical acidosis complicated by guanidine intoxication.

SUMMARY

Guanidine intoxication has been shown to cause an acidosis due primarily to increased production and defective metabolism of lactic acid. The administration of alkalinizing sodium salts in the treatment of this acidosis is attended by unusual difficulties because of the danger associated with any temporary decrease in the effectiveness of calcium ions in the presence of increased guanidine. Sodium lactate fails to increase the alkali reserve because of the inability of the subject to utilize lactates. Sodium bicarbonate while at times both dangerous and ineffective if used alone, can be made safe and promptly efficient if calcium medication is combined with its administration. A very gradual relief of acidosis results from calcium medication alone or from the intravenous administration of fluid in the form of glucose solution or normal saline. The latter procedure also improves the condition of the subject by hastening the excretion of guanidine through improved kidney function. We have found the most satisfactory treatment of the acidosis produced by the administration of guanidine to be the use of sodium bicarbonate combined with repeated intravenous calcium medication and the injection of liberal amounts of fluids to increase the urinary output. The same form of treatment is recommended for severe acidosis in clinical subjects with hyperguanidinemia.

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THE EFFECT OF ALKALI ON THE ABSORPTION OF A PEPTIDE OF THYROXINE FROM THE GASTRO-INTESTINAL TRACT

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When pure thyroxine suspended in distilled water is administered by mouth it has only a slight effect on the basal metabolism (1). However, if pure thyroxine is dissolved in an excess of sodium hydroxide (presumably forming the disodium salt of thyroxine (2)) and is then administered by mouth, there is a well-marked increase in the basal metabolism which, on the average, is 63 per cent as great as the increase which follows the intravenous administration of thyroxine in the same form (3, 4). If the monosodium salt of thyroxine (tablets) is administered by mouth, the increase in basal metabolism is only 22 per cent as great as that which follows the intravenous injection of an equivalent dose of thyroxine in alkaline solution (1, 5). Thus an alkaline solution of thyroxine is nearly three times as effective, on the average, as an equivalent dose of the mono-sodium salt when both compounds are administered by mouth. Further-more, it has been noted that the increase in basal metabolism produced by the oral administration of single large doses of thyroxine in alkaline solution is, on the average, about the same as that produced by single large doses of desiccated thyroid containing the same amount of iodine (6, 7).

By proteolytic digestion of thyroid, Harington and Salter (8) obtained a peptide of thyroxine which they state has "a much wider range of solubility" than thyroxine and contains thyroxine in a levorotatory form. Salter, Lerman and Means (9) have recently reported that this peptide had about the same effect whether given orally or intravenously and that it had the same effect as racemic thyroxine when both were administered intravenously in doses which contained the same total amounts of iodine. They have kindly informed us that, for oral administration, their peptide "was dissolved in sodium hydroxide" and the solution "then neutralized with phosphoric acid."

It seemed desirable to compare the effect of oral administration of this peptide with that of an equivalent amount of thyroxine in alkaline solution.

Accordingly, one of us (S. B. N.) prepared a similar substance by the method of Harington and Salter (8), using double the concentration of enzymes employed by them, i.e. 1.0 per cent pepsin and 0.4 per cent trypsin. From 1620 grams of desiccated thyroid [1] there were obtained 310 mgm. of a light buff colored powder containing 48 per cent iodine by the method of Leland and Foster (10) and 2.5 per cent total nitrogen by the micro-Kjeldahl method, giving a nitrogen:iodine ratio of 0.48:1. Before hydrolysis, 43 per cent of the total nitrogen was found to be in the amino form by the method of Folin (11); and, after six hours hydrolysis by the method of Harington and Salter (8), 78 per cent was found to be in this form. Most of the digestion products obtained by Harington and Salter contained from 45 to 50 per cent iodine and 5 per cent nitrogen, giving a nitrogen:iodine ratio of 1:1, but two of their products gave nitrogen: iodine ratios of 0.6:1. The product used by Salter, Lerman and Means (9) contained 49 per cent iodine and 3.3 per cent nitrogen, giving a nitrogen:iodine ratio of 0.6:1. When subjected to the action of nitrous acid and made alkaline with ammonia, the product we obtained gave an orange pink color in contrast to the red pink color which is characteristic of thyroxine. When it was dissolved in N/10 sodium hydroxide, the solution had a slight yellow tinge. In order to eliminate the possibility that it contained acid-soluble iodine, a small amount was dissolved in alkaline solution and precipitated at pH 5.0 by the cautious addition of dilute hydrochloric acid. Since 95 per cent of the iodine was recovered in the precipitate, it seemed logical to conclude that diiodotyrosine and inorganic iodine were both absent. From these data it would appear that our product is similar to the thyroxine peptide described by Harington and Salter.

Our peptide, like pure thyroxine, was insoluble in distilled water but was soluble in alkali. In view of this similarity in solubility it seemed all the more desirable to compare the calorigenic effects of oral administration of the two substances, (a) when suspended in distilled water, and (b) when dissolved in alkaline solution.

METHOD

The observations were made on three patients with well-marked myxedema. In the second patient the myxedema was spontaneous and in the other two it followed a subtotal thyroidectomy for exophthalmic goiter. Parts of the data (exclusive of those on the peptide) have been published elsewhere, as collected, to illustrate other points (1, 3, 7). The Sanborn-Benedict machine was used in the determinations of basal metabolism and

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[1] The desiccated thyroid, pepsin and trypsin used in the preparation of this substance were very kindly supplied by Dr. Klein of the Wilson Laboratories, Chicago.

Aub-DuBois standards in the calculations. The number of calories produced by each type of treatment ("excess calories") has been calculated by a method previously described (3, 12). The synthetic thyroxine used was the crystalline powder purchased from Hoffmann-La Roche. The monosodium salt of synthetic thyroxine was bought from the same manufacturers in the form of tablets, each of which contained 1.03 mgm. of the salt.

<div align="center">DATA</div>

The data are recorded in Charts 1 to 3 and summarized in Tables I and II. To facilitate comparisons in the tables, the effects of thyroxine have been calculated in terms of 10 mgm. (6.5 mgm. of iodine) and the effects of the peptide in terms of 13.5 mgm. (6.5 mgm. of iodine). It may be seen from Table I that, regardless of whether the effects of the various types of treatment are compared on the basis of the amount of increase in the basal metabolism or on the basis of the number of calories produced, similar conclusions are arrived at for the three patients in this study. Therefore, for the sake of simplicity, in discussing the data we shall confine our attention almost entirely to the amount of increase in the basal metabolism.

It may be noted that when the peptide was given by mouth suspended in distilled water, it had only about one-third as much effect as when it was given in alkaline solution. Thus, by calculation, the average increase in basal metabolism for a dose of 13.5 mgm. containing 6.5 mgm. of iodine was from minus 31 per cent to minus 22 per cent when the peptide was given suspended in distilled water and from minus 32 per cent to minus 7 per cent when it was given in alkaline solution. These increases in metabolism are nearly the same as those produced respectively by the oral administration of the monosodium salt of thyroxine in tablet form (from minus 33 per cent to minus 23 per cent, on the average) and by the oral administration of thyroxine in alkaline solution (from minus 32 per cent to minus 10 per cent, on the average) in doses which contained the same amounts of iodine. No adequate explanation can be offered for the similarity in the effect of oral administration of the peptide suspended in distilled water and that of the monosodium salt of thyroxine. It would appear that the peptide, when given suspended in distilled water, is absorbed as well as the monosodium salt in tablet form. If absorption depends upon the formation of a soluble salt in the small intestine, then it must follow that the peptide forms a soluble salt in this portion of the gastro-intestinal tract with greater ease than pure thyroxine, because, in the same dose by mouth, thyroxine as the free amino-acid does not produce a definite effect on the basal metabolism (1). It is possible that the effect of administering the peptide by mouth in an alkaline solution is slightly greater than that of administering thyroxine by mouth in an alkaline solution, but the data are not extensive enough to settle this point.

TABLE I

Comparison of calorigenic effects of thyroxine peptide with those of thyroxine and desiccated thyroid

Patient	Medication	Total iodine content of substance used	Basal metabolic rate before medication	Level to which basal metabolic rate rose	Change in basal metabolic rate	Time required for maximum change in basal metabolic rate	Length of time basal metabolic rate was affected	Time occupied by descending portion of metabolism curve	Number of squares (from charts for calculating "excess calories")	Number of "excess calories" produced	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution	
											On basis of increase in basal metabolic rate	On basis of "excess calories" produced
		mgm.	per cent normal	per cent normal	points	days	days	days			per cent	per cent
Mrs. B. G. Lab. No. 2767	10 mgm. synthetic thyroxine in form of its monosodium salt (tablets) by mouth	6.5	−37	−22	15	8	50	28	426	6,485		
	10 mgm. synthetic thyroxine in form of its monosodium salt (tablets) in alkaline solution by mouth	6.5	−34	−17	17	6	52	32	580	8,910		
	50 mgm. pure synthetic thyroxine suspended in distilled water by duodenum	32.5	−32	−29	3	6	15	4	31	485		
	Calculated effect of 10 mgm. pure thyroxine suspended in distilled water by duodenum	6.5	−32	−31	1					97		
	10 mgm. synthetic thyroxine in alkaline solution by mouth	6.5	−34	−10	24	8	62	48	837	13,025		
	14.4 mgm. thyroxine peptide suspended in distilled water by mouth	6.9	−33	−22	11	3	41	30	282	4,410		
	Calculated effect of 13.5 mgm. thyroxine peptide suspended in distilled water by mouth	6.5	−33	−23	10					4,135		
	13.5 mgm. thyroxine peptide in alkaline solution by mouth	6.5	−36	− 8	28	8	54	43	677	10,535		

TABLE I (continued)

Patient	Medication	Total iodine content of substance used	Basal metabolic rate before medication	Level to which basal metabolic rate rose	Change in basal metabolic rate	Time required for maximum change in basal metabolic rate	Length of time basal metabolic rate was affected	Time occupied by descending portion of metabolism curve	Number of squares (from charts for calculating "excess calories")	Number of "excess calories" produced	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution	
											On basis of increase in basal metabolic rate	On basis of "excess calories" produced
		mgm.	per cent normal	per cent normal	points	days	days	days			per cent	per cent
Mrs. M. K. Lab. No. 200	10 mgm. synthetic thyroxine in alkaline solution intravenously	0.5	−20	−1	25	3	20	23	408	5,135		
	10 mgm. Squibb's thyroxine in alkaline solution intravenously	0.5	−23	−4	19	4	20	17	312	4,300		
	Average effect of 10 mgm. thyroxine in alkaline solution intravenously	0.5	−25	−3	22	3	20	20		4,720	100	100
	10 mgm. synthetic thyroxine in form of the monosodium salt (Squibb) by mouth	0.5	−25	−19	6	3	25	11	102	1,303	27	28
	10 mgm. synthetic thyroxine in alkaline solution by mouth	0.5	−20	−6	20	6	33	21	332	4,315	91	91
	2.75 grams (12.6 grains) desiccated thyroid (tablets) by mouth	0.3	−20	−11	15	3	33	20	218	3,170	68	69
	Calculated effect of 2.75 grams desiccated thyroid by mouth	0.3	−20	−11	15					3,200		
	13.4 mgm. thyroxine peptide suspended in distilled water by mouth	0.0	−20	−17	6	2	20	17	111	1,455	36	29
	Calculated effect of 13.5 mgm. thyroxine peptide suspended in distilled water by mouth	0.3	−20	−19	8					1,305		
	13.5 mgm. thyroxine peptide in alkaline solution by mouth	0.5	−29	−6	23	3	31	21	345	4,600	100	99

TABLE I (continued)

Patient	Medication	Total iodine content of substance used	Basal metabolic rate before medication	Level to which basal metabolic rate rose	Change in basal metabolic rate	Time required for maximum change in basal metabolic rate	Length of time basal metabolic rate was affected	Time occupied by descending portion of metabolism curve	Number of squares (from charts for calculating "excess calories")	Number of "excess calories" produced	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution	
											On basis of increase in basal metabolic rate	On basis of "excess calories" produced
		mgm.	per cent normal	per cent normal	points	days	days	days			per cent	per cent
Mrs. A. R. Lab. No. 1000	7.5 mgm. synthetic thyroxine in alkaline solution intravenously	4.9	−30	− 7	23	6	45	32	476	6,910		
	7.5 mgm. Squibb's thyroxine in alkaline solution intravenously	4.9	−34	− 9	25	7	53	41	623	9,000		
	Average effect of 7.5 mgm. thyroxine in alkaline solution intravenously	4.9	−32	− 8	24	6	49	37		7,955	"	
	Calculated effect of 10 mgm. thyroxine in alkaline solution intravenously	6.5	−32	0	32					10,605	100	100
	7.5 mgm. synthetic thyroxine in form of its monosodium salt (tablets) by mouth	4.9	−36	−29	7	6	27	10	125	1,825		
	Calculated effect of 10 mgm. synthetic thyroxine in form of its monosodium salt by mouth	6.5	−36	−27	9					2,435	28	23
	7.5 mgm. synthetic thyroxine in alkaline solution by mouth	4.9	−35	−19	16	5	42	28	342	4,990		
	Calculated effect of 10 mgm. synthetic thyroxine in alkaline solution by mouth	6.5	−35	−14	21					6,655	60	63
	2.05 grams (31.7 grains) desiccated thyroid (tablets) by mouth	4.7	−35	−21	14	2	34	31	209	4,385		
	Calculated effect of 2.83 grams desiccated thyroid by mouth	0.5	−35	−16	10					6,055	59	57
	10.9 mgm. thyroxine peptide suspended in distilled water by mouth	5.2	−34	−26	8	1	28	26	149	2,205		
	Calculated effect of 13.5 mgm. thyroxine peptide suspended in distilled water by mouth	6.5	−34	−24	10					2,730	31	26
	10.2 mgm. thyroxine peptide in alkaline solution by mouth	4.9	−34	−14	20	3	47	42	500	7,455		
	Calculated effect of 13.5 mgm. thyroxine peptide in alkaline solution by mouth	0.5	−34	− 8	20					9,865	81	93

TABLE I (continued)

Patient	Medication	Total iodine content of substance used (mgm.)	Basal metabolic rate before medication (per cent normal)	Level to which basal metabolic rate rose (per cent normal)	Change in basal metabolic rate (points)	Time required for maximum change in basal metabolic rate (days)	Length of time basal metabolic rate was affected (days)	Time occupied by descending portion of metabolism curve (days)	Number of squares (from charts for calculating "excess calories")	Number of "excess" calories produced	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution — On basis of increase in basal metabolic rate (per cent)	Change in terms of response to intravenous injection of 10 mgm. of thyroxine in alkaline solution — On basis of "excess calories" produced (per cent)
	Average											
	10 mgm. synthetic thyroxine in form of its mono-sodium salt (tablets) by mouth											
	All three patients	0.5	−33	−23	10					3,110	30	21
	Last two patients	0.5	−31	−23	9					1,870		
	10 mgm. synthetic thyroxine in alkaline solution by mouth											
	All three patients	0.5	−33	−10	22					8,090	78	72
	Last two patients	0.5	−31	−10	21					5,483		
	11.5 mgm. thyroxine peptide suspended in distilled water by mouth											
	All three patients	0.5	−31	−22	9					2,715	33	27
	Last two patients	0.5	−30	−21	9					2,050		
	11.5 mgm. thyroxine peptide in alkaline solution by mouth											
	All three patients	0.5	−32	−7	25					8,305	89	63
	Last two patients	0.5	−31	−7	24					7,290		
	7.45 grams desiccated thyroid (tablets) by mouth											
	Last two patients	0.5	−31	−11	17					4,060	63	61
	10 mgm. thyroxine alkaline solution intravenously											
	Last two patients	0.5	−29	−2	27					7,065	100	100

TABLE II

Summary of effects of various compounds of thyroxine

Medication	Total iodine content of substance used	Number of patients	Number of administrations	Average basal metabolic rate before treatment	Average level to which basal metabolic rate rose	Average change in basal metabolic rate	Average number of "excess calories" produced	Change in terms of average response to intravenous injection of 10 mgm. thyroxine in alkaline solution	
								On basis of increase in basal metabolic rate	On basis of "excess calories" produced
	mgm.			*per cent normal*	*per cent normal*	*points*		*per cent*	*per cent*
10 mgm.[1] pure synthetic thyroxine suspended in distilled water, by duodenum..........	6.5	3	3	−30	−29	1		3	
10 mgm.[2] pure synthetic thyroxine suspended in distilled water, by mouth..........	6.5	4	4	−32	−30	2		6	
10 mgm.[3] synthetic thyroxine in form of monosodium salt (tablets), by mouth..........	6.5	6	6	−28	−21	7		22	
Patients receiving monosodium salt by mouth, in whom "excess calories" were calculated..........	6.5	4	4	−30	−22	8	2,755	25	18
13.5 mgm. thyroxine peptide suspended in distilled water, by mouth..........	6.5	3	3	−31	−22	9	2,745	28	18
10 mgm.[4] synthetic thyroxine in alkaline solution, by mouth..........	6.5	5	5	−31	−11	20		63	
Patients receiving thyroxine in alkaline solution by mouth, in whom "excess calories" were calculated..........	6.5	4	4	−34	−12	22	9,010	69	58
2.83 grams[5] desiccated thyroid (tablets) by mouth..........	6.5	5	5	−37	−15	22	7,405	69	48
13.5 mgm. thyroxine peptide in alkaline solution, by mouth.....	6.5	3	3	−32	− 7	25	8,365	78	54
10 mgm.[6] thyroxine in alkaline solution (sodium or potassium hydroxide), intravenously..........	6.5	6	8	−37	− 5	32	15,520	100	100

[1] The doses used were 10 mgm., 40 mgm. and 50 mgm.   [2] The doses used were 10 mgm., 30 mgm., 40 mgm. and 100 mgm.
[3] The doses used were four of 10 mgm. each, one of 30 mgm. and one of 40 mgm.
[4] All doses were 10 mgm. except one of 7.5 mgm.   [5] All doses were 2.75 grams except one of 2.05 grams.
[6] All doses were 10 mgm. except two of 7.5 mgm.   Sodium hydroxide was used to dissolve the thyroxine for all administrations except one, in which potassium hydroxide was used.
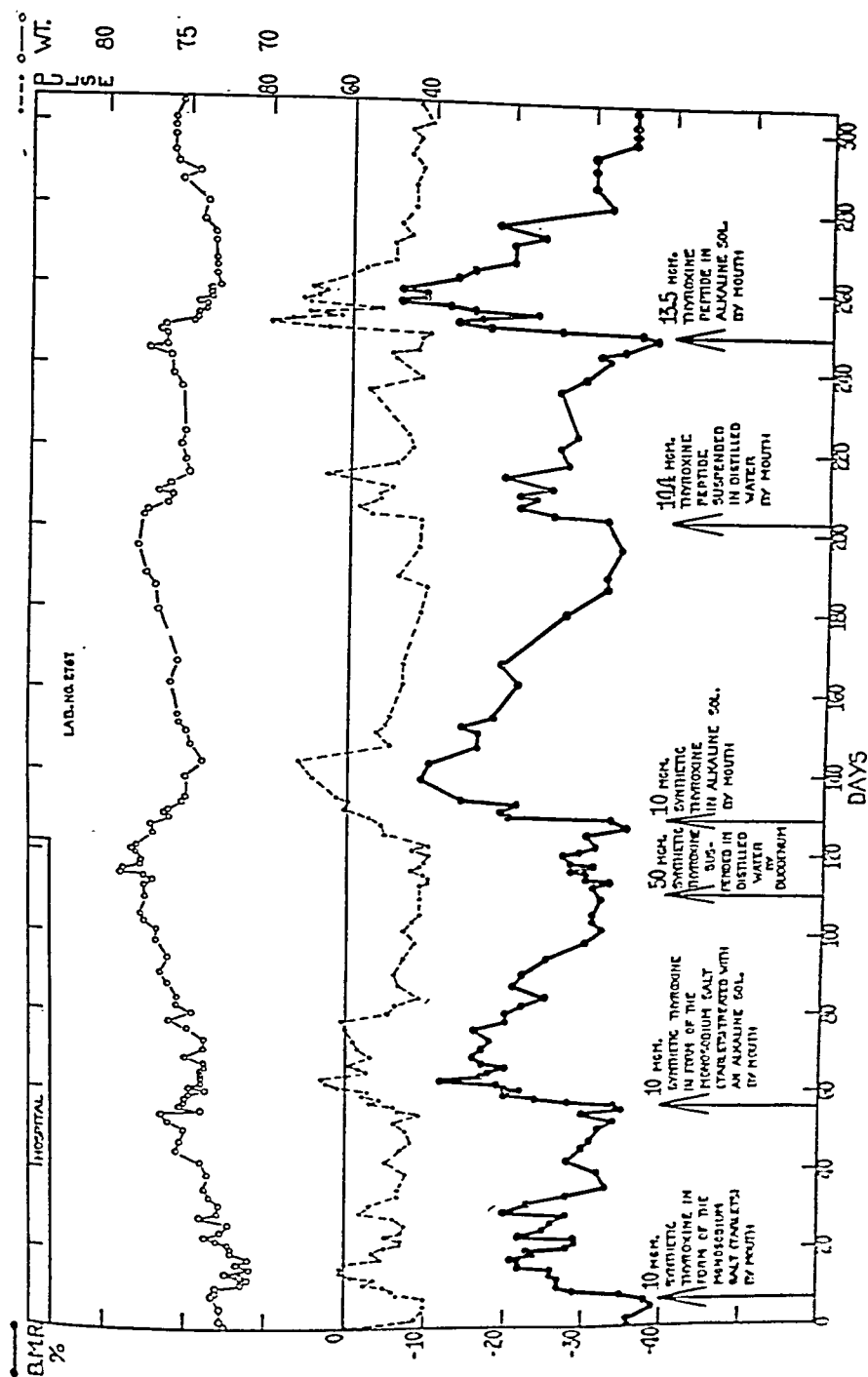
CHART 1. MRS. B. G. HEIGHT 158 CM. AGE 38

Comparison of the effects of oral administration of thyroxine peptide suspended in distilled water and in alkaline solution with those of administering thyroxine in various forms by mouth, and intravenously in alkaline solution.

For details of the administrations of pure thyroxine and of its sodium salts, see another publication (1). The single dose of 111 mgm. of thyroxine peptide was suspended in distilled water and administered by mouth at 2.35 p.m, May 26, 1933, a total of 500 cc. of distilled water being used, largely for rinsing. The patient had had no breakfast or lunch. The single dose of 113 mgm. of thyroxine peptide in alkaline solution was administered by mouth at 2.25 p.m, July 11, 1933, a total of 10 drops of 10 per cent sodium hydroxide and 500 cc. of distilled water being used for solution and rinsing. The patient had had no breakfast or lunch.

CHART 2. MRS. M. K. HEIGHT 152 CM. AGE 36

Comparison of the effects of oral administration of thyroxine peptide suspended in distilled water and in alkaline solution with the effects of administering thyroxine in various forms by mouth, and intravenously in alkaline solution.

For details of the various administrations of monosodium thyroxine, thyroxine in alkaline solution, and desiccated thyroid, see other publications (3, 7). The single dose of 14.4 mgm. of thyroxine peptide was administered by mouth at 2.35 p.m, May 23, 1933, suspended in distilled water, a total of 250 cc. of water being used, largely for rinsing. The single dose of 13.5 mgm. of thyroxine peptide in alkaline solution was administered by mouth at 3.50 p.m., June 20, 1933, a total of 14 drops of 10 per cent sodium hydroxide and 250 cc. of distilled water being used for solution and rinsing. Preceding both administrations of the peptide, the patient had not eaten breakfast but had eaten lunch at 12 o'clock.

CHART 3. MRS. A. R. HEIGHT 160 CM. AGE 33

Comparison of the effects of oral administration of thyroxine peptide suspended in distilled water and in alkaline solution with those of administering thyroxine in various forms by mouth, and intravenously in alkaline solution.

For details of the various administrations of monosodium thyroxine, thyroxine in alkaline solution, and desiccated thyroid, see other publications (3, 7). The single dose of 10.9 mgm. of thyroxine peptide was administered by mouth, suspended in distilled water, at 12 o'clock (noon), May 29, 1933, a total of 500 cc. of water being used, largely for rinsing. The single dose of 10.2 mgm. of thyroxine peptide in alkaline solution was administered by mouth at 3.35 p.m., June 28, 1933, a total of 9 drops of 10 per cent sodium hydroxide and 500 cc. of distilled water being used for solution and rinsing. Preceding both administrations of the peptide, the patient had not eaten breakfast or lunch.

their polypeptide would have produced an increase in the basal metabolism from minus 36 per cent to minus 6 per cent on the average, a change which is nearly the same as that produced by the oral administration of 6.5 mgm. of iodine in the form of our peptide (13.5 mgm.) in alkaline solution. We have compared our data with theirs in Charts 4 and 5. It is of interest that their peptide was dissolved in an alkaline solution and the solution then neutralized.

It is of interest to combine the data of the present study with those which we have previously reported concerning the effects of the administration of thyroxine in various forms by the oral and intravenous routes. This has been done in Table II. It may be noted that, on the average, the increases in basal metabolism produced by the oral administration of single large doses of monosodium thyroxine, thyroxine peptide suspended in distilled water, pure thyroxine in alkaline solution, desiccated thyroid and thyroxine peptide in alkaline solution are respectively 22 per cent, 28 per cent, 63 per cent, 69 per cent and 78 per cent as great as those produced by the intravenous injection of an alkaline solution of single large doses of pure thyroxine containing the same amount of iodine. In terms of " excess calorie " production, the corresponding figures are 18, 18, 58, 48 and 54 per cent respectively.

SUMMARY

From a proteolytic digest of desiccated thyroid we have prepared a peptide of thyroxine containing 48 per cent iodine, with a nitrogen : iodine ratio of 0.48 : 1. This product is insoluble in distilled water but soluble in a dilute solution of sodium hydroxide.

When suspended in distilled water and administered by mouth to patients with myxedema it produced only a slight increase in the basal metabolism, which was about the same as that produced by oral administration of the monosodium salt of thyroxine in doses which contained the same amounts of iodine, and about one-quarter as great as that produced by thyroxine in alkaline solution given intravenously. However, when administered by mouth in an alkaline solution, the peptide produced a well-marked increase in basal metabolism which was nearly four-fifths as great as that produced by thyroxine in alkaline solution given intravenously, and slightly greater than those produced by oral administration of desiccated thyroid and thyroxine in alkaline solution.

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FACTORS DETERMINING THE EFFECT OF EXERCISE ON BLOOD SUGAR IN THE DIABETIC

By RUSSELL RICHARDSON

WITH THE TECHNICAL ASSISTANCE OF

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The work of Lawrence (1), Hetzel and Long (2) and others has established certain facts regarding the effect of exercise on blood sugar in diabetic individuals. It seemed desirable, however, to examine a larger number of such persons by means of a standard form of exercise to determine whether diabetics differ in their response to exercise and whether the severity of the diabetes influences the response. It seems certain that carbohydrate exchange is accelerated during exercise. Such acceleration should accentuate deficiencies which might pass unnoticed during the resting state.

It is commonly believed that exercise reduces the blood sugar in diabetics, though as yet too few patients have been studied to establish this as a fact. Former studies on the effects of exercise in diabetes have usually been made on a small number of patients and with a form of exercise which does not lend itself to definite measurement and standardization. Moreover, some of the forms used have been so exhausting in character as to be not suitable for the more severe diabetics. Exercise, to be most appropriate for such a study as this, must be sufficient to affect the blood sugar of normal persons and of diabetic patients in good condition and yet must not be too strenuous for those diabetic patients who may be in poor condition.

The exercise selected consisted of four five-minute periods of work on the ordinary type of rowing machine exerciser. By the use of either two or three springs a pull of 50 to 75 pounds could be provided. The rate of stroke was kept constant at forty per minute with the aid of a metronome. Between successive five-minute periods of exercise was interposed a two-minute period of rest, during which the patient remained seated on the machine. The patients were taught to flex the legs and back but not the arms. The blood for sugar determinations was taken from the arms. The external work of the summation of the pulling strokes for each half hour period was approximately 4.5×10^6 ergs with two springs
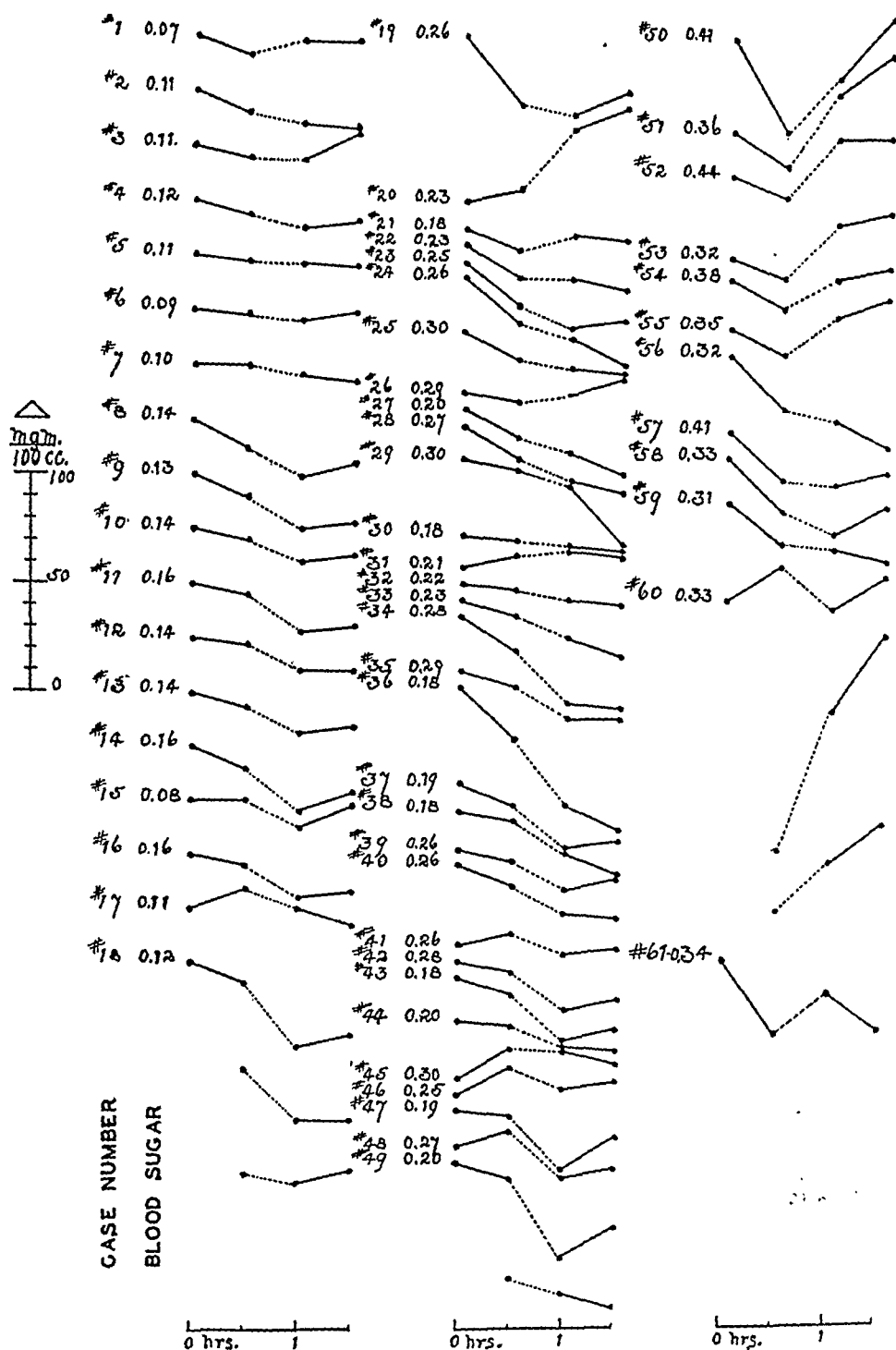
FIG. 1. SHOWING BLOOD SUGAR CURVES OF 61 DIABETICS WITH STANDARDIZED FORM OF EXERCISE

———— = rest — — — —. = exercise.

and 6.8×10^5 with three springs. This exercise was found sufficient to affect the blood sugar and yet was not too exhausting for even poorly nourished patients. The subjects rested one half hour on a bed adjacent to the rowing machine immediately before and after the exercise. Neither food nor insulin had been taken for 16 hours. Blood for determination of sugar was drawn from a vein at the elbow at the beginning of the first rest period, before and after the exercise and after the second rest period. Blood sugar was measured by Benedict's method. The patients were from the metabolic clinic.

In Figure 1 are shown the blood sugar curves for 61 patients exercised in this manner. The curves are divided into three groups according to the fasting blood sugar. The first group consists of those from patients
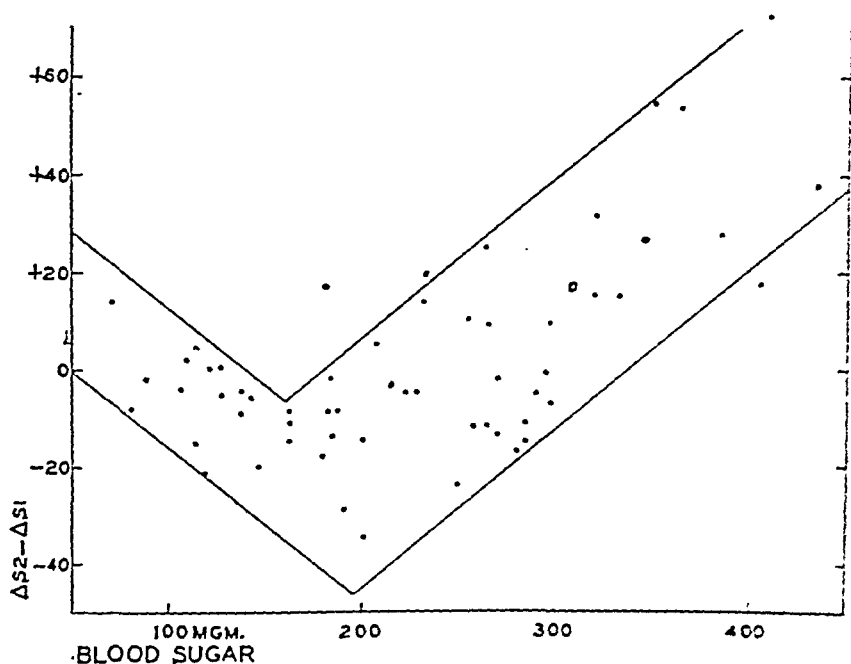
FIG. 2a. SHOWING THE ALTERATION DURING EXERCISE IN THE RATE OF BLOOD SUGAR CHANGE IN MILLIGRAMS PER HALF HOUR PLOTTED AGAINST THE FASTING BLOOD SUGAR.

in whom the fasting blood sugar was below 175 mgm. per 100 ml. of blood, the second group from those in whom the blood sugar lay between 175 mgm. and 300 mgm. and the third group from those in whom it was over 300 mgm. The curves are arranged in each column in the order of the change in the slope of the blood sugar curve brought about by exercise. This change in slope is expressed as $\Delta S2 - \Delta S1$ in which $\Delta S1$ equals the change in blood sugar during the half hour of rest preceding the exer-

cise and $\Delta S2$ the change in blood sugar during the half hour of exercise. A more rapid rise or less rapid fall in blood sugar results in a positive difference in slope.

In the first group the result of the exercise was predominantly a slight change in slope downward. This decrease within this group appears to be slightly greater as the fasting blood sugar was higher. In the second group, exercise induced with about equal frequency an upward or downward inflection in the course of the curve. In the third group there was an upward inflection in all patients except one. These differences are shown in Figure 2a, in which the change in the slope of the blood sugar curve is plotted against the fasting blood sugar. It will be more evident here that whereas below a fasting blood sugar of 175 mgm. the higher the blood sugar the greater the downward inflection in the curve as a result of exercise, above this fasting level the higher the fasting blood sugar the greater was the tendency to upward inflection as a result of exercise.

It appears that in the severe diabetic under the conditions of these experiments there is a tendency toward increased blood sugar as a result of exercise. This was true whether the high fasting hyperglycemia was
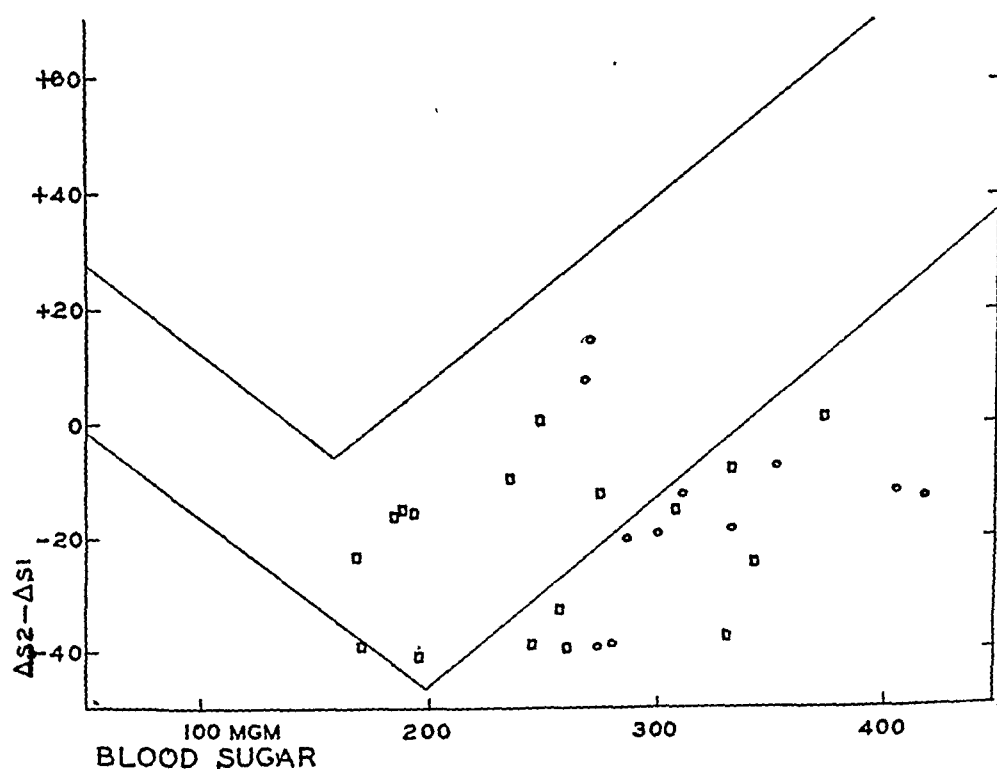
FIG. 2b. SHOWING THE ALTERATION IN RATE OF BLOOD SUGAR CHANGE DURING EXERCISES DONE LATE IN THE DAY AS SHOWN IN FIGURES 6 AND 7.

The square and circle in Figure 2a show the result of exercise in the same patients without food or insulin.

the result of a permanently severe diabetes or only a temporary condition consequent upon inadequate therapy. The patients in the former category, namely, all of those in Group 3 except the last, had been adjusted on diet and insulin and were in good condition at the time of the test. The fasting hyperglycemia had been developed as a result of 16 hours without insulin.
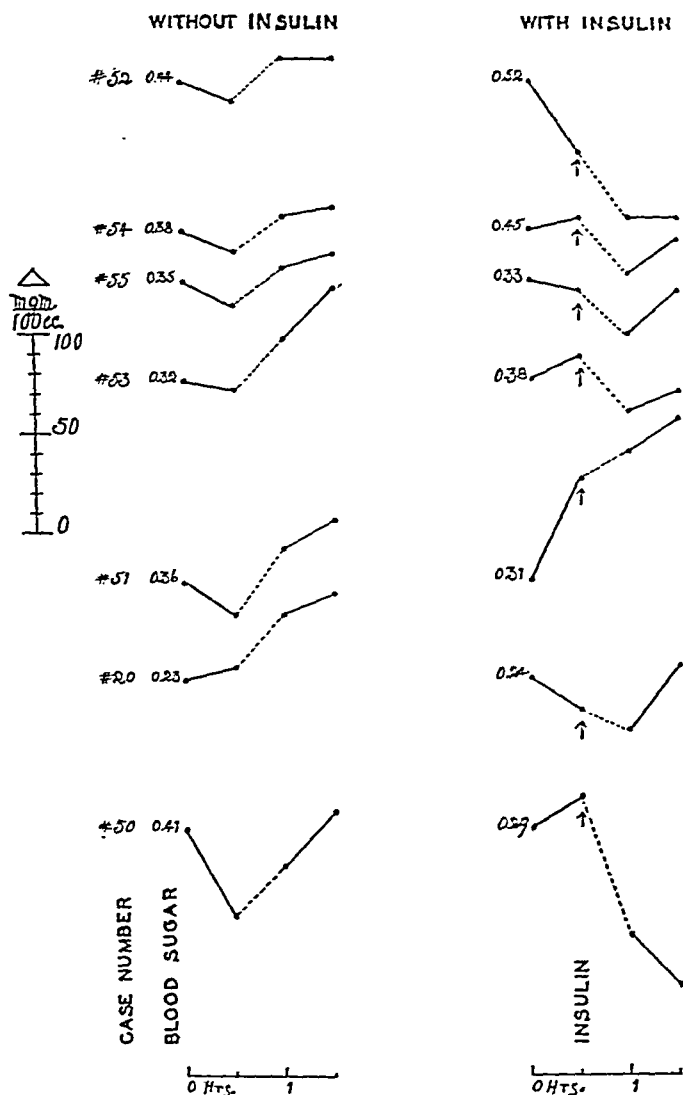
FIG. 3. SHOWING EFFECT OF 0.1 UNIT OF INSULIN GIVEN INTRAVENOUSLY IMMEDIATELY BEFORE THE EXERCISE

The curves in the left hand column were done without insulin.

——————— = rest   — — — — = exercise.

| indicates 0.1 unit of insulin.

Four patients with marked hyperglycemia on their first visit to the clinic were exercised at this visit while they were still in poor clinical condition. The blood sugar of all increased during exercise at this time. After they had been adjusted on a suitable diet with insulin, so that their fasting blood sugar was less than 120 mgm., exercise was then accompanied by a fall in blood sugar.

The use of insulin intravenously immediately before the exercise, even in very small doses, was followed by an alteration in the behavior of the blood sugar curve during exercise. In the experiments demonstrating this, variation in absorption of the insulin was eliminated by employing the intravenous route of administration; 0.1 unit was given from 2 to 3 minutes before the exercise. This amount of insulin, when not followed by exercise, had not been sufficient to cause any marked change in blood sugar. When such a dose of insulin was given to patients, who without insulin exhibited an increase in blood sugar during exercise, there resulted a definite decrease in the blood sugar during exercise. In Figure 3 are shown the blood sugar curves of seven patients exercised, first without insulin and then with insulin. It is evident that even a very small amount of insulin given intravenously immediately before exercise influenced the course of the blood sugar curve. In Case 51 it will be noted that in the experiment with insulin, though the blood sugar was slightly higher after exercise than before, still it was lower than would have been expected after rest or exercise without insulin.

It is interesting to contrast with these experiments the effect of five times the dose of insulin, given intravenously but given 30 minutes before commencing the exercise, as shown by the fourth curves from Cases 59 and 55 in Figure 4. In these experiments 0.5 unit of insulin was given intravenously after ½ hour rest; the exercise, begun 30 minutes later, was in these experiments accompanied by a rising blood sugar. Further studies are in progress as to the significance of the difference between these two groups of experiments. When 5 units of insulin were given subcutaneously and the patient fed fillers, and exercise was begun 60 minutes later, a fall in blood sugar occurred (see first curves of Figures 6 and 7). It is evident that not only the size of the dose but the mode of administration, whether intravenous or subcutaneous, and the time of its administration in relation to the exercise must be taken into account.

In order to investigate the relative effectiveness of insulin combined with rest or with exercise, 8 patients, whose blood sugar had increased or had remained unchanged following exercise, were given 0.5 unit of insulin intravenously followed by 60 minutes of rest and were then given a second 0.5 unit of insulin intravenously followed immediately by the exercise. In five of these patients the decrease in blood sugar was greater after the second dose of insulin with the exercise whereas in three it was greater
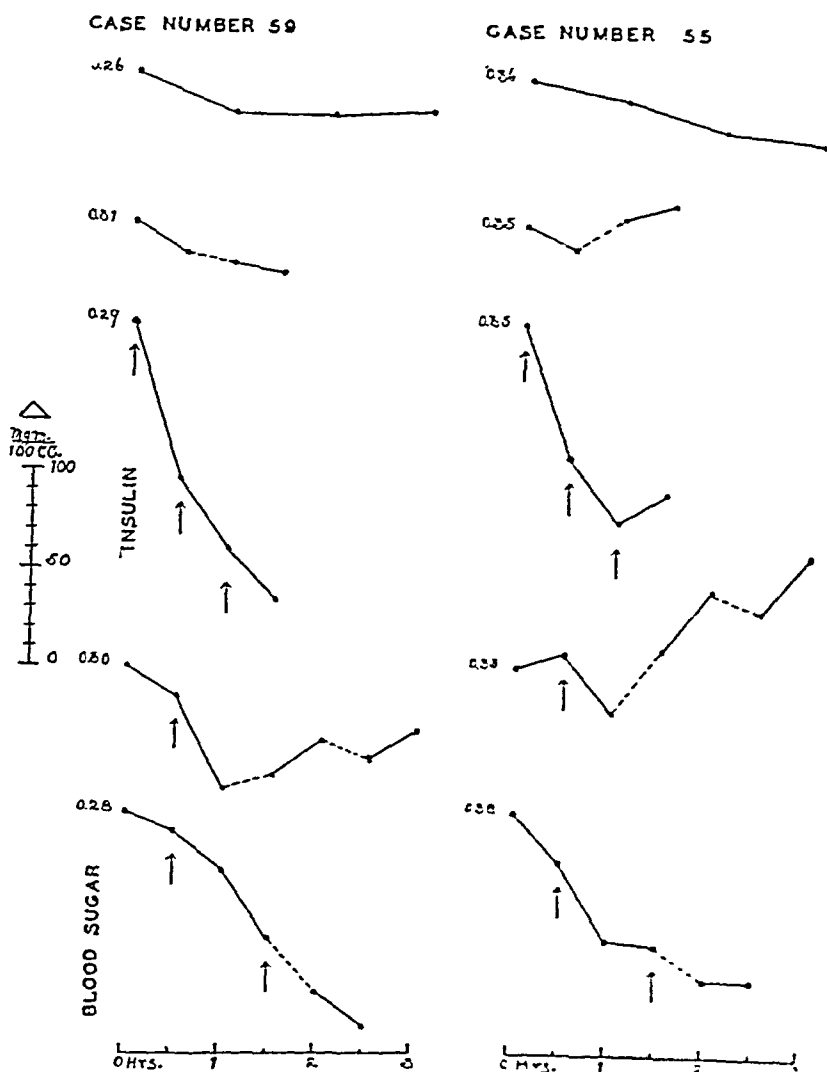
FIG. 4. SHOWING EFFECT ON BLOOD SUGAR OF VARIOUS COMBINATIONS OF REST, EXERCISE AND INSULIN

―――――― = rest   ― ― ― ― = exercise.
↑ indicates 0.5 unit of insulin given intravenously.

after the first dose of insulin which was followed by rest (see Figure 5). In the former group the average decrease in blood sugar was 13 mgm. during ½ hour of rest and 33 mgm. during ½ hour of exercise. In the latter group the average resting decrease in blood sugar was 44 mgm. and the decrease during exercise was 25 mgm. There was no consistent effect of either rest or exercise on the blood sugar curve after this amount of insulin in these severe diabetics.

The first curves from each patient confirm the observation previously made, that during rest without food or insulin there is little change in blood sugar.

It will be noted that there is a considerable similarity in blood sugar changes at corresponding periods on different days; thus the resting periods in curves number 1, 2, 4, and 5 are quite consistent, as also the responses to insulin during the second periods in curves number 3, 4, and 5.

In order to study the effect of food in association with insulin and exercise a longer experiment was performed which included insulin, two meals and four periods of exercise, the whole extending over 8½ hours. These experiments also were carried out on Cases 59 and 55. The food was varied, including a day with a low intake, a day with an average diabetic diet and one day each on which protein, carbohydrate and fat respectively comprised the bulk of the diet. Five units of insulin were given subcutaneously on each day except two. On one of these no insulin was given and on the other 5 units were given intravenously. In Figures 6 and 7 are shown the blood sugar curves for these days.

Several points are worthy of note. The similarity in response to intravenous and subcutaneous insulin is evident. Protein and fat seemed to provide no immediately available effect to offset the action of the insulin. The exercises later in the day are of interest. It had been shown that these patients when fasting had an increased blood sugar after exercise. The decrease in blood sugar with all periods of exercise later in the day in these experiments suggests either that some of the small dose of insulin given at the beginning of the day still remained available or that food intake acted in some way to reduce blood sugar during exercise. On the last day no insulin was given and yet the same reduction in blood sugar occurred. This fact seems to show that the insulin given in the morning was not the deciding factor. It appears that intake of food provided a condition which, with exercise, resulted in reduction of blood sugar (see Figures 6 and 7, curves 7, and Figure 2b).

DISCUSSION

The antagonistic action of insulin and adrenalin in controlling blood sugar is established. There are, in addition, two other factors which may at times affect this control so that, in all, four factors may be said to govern the blood sugar in exercise. These are: (1) Adrenalin raising the blood sugar. (2) Insulin lowering the blood sugar. (3) The amount of glycogen in the muscles as affected by the exercise itself—" muscle glycogen vacuum " so called. (4) The amount of glycogen in the liver available for supplying sugar to the blood.

In the normal individual exercise reduces the amount of glycogen in the muscles and there is created a demand for glucose at this site. This

CASE NUMBER 59
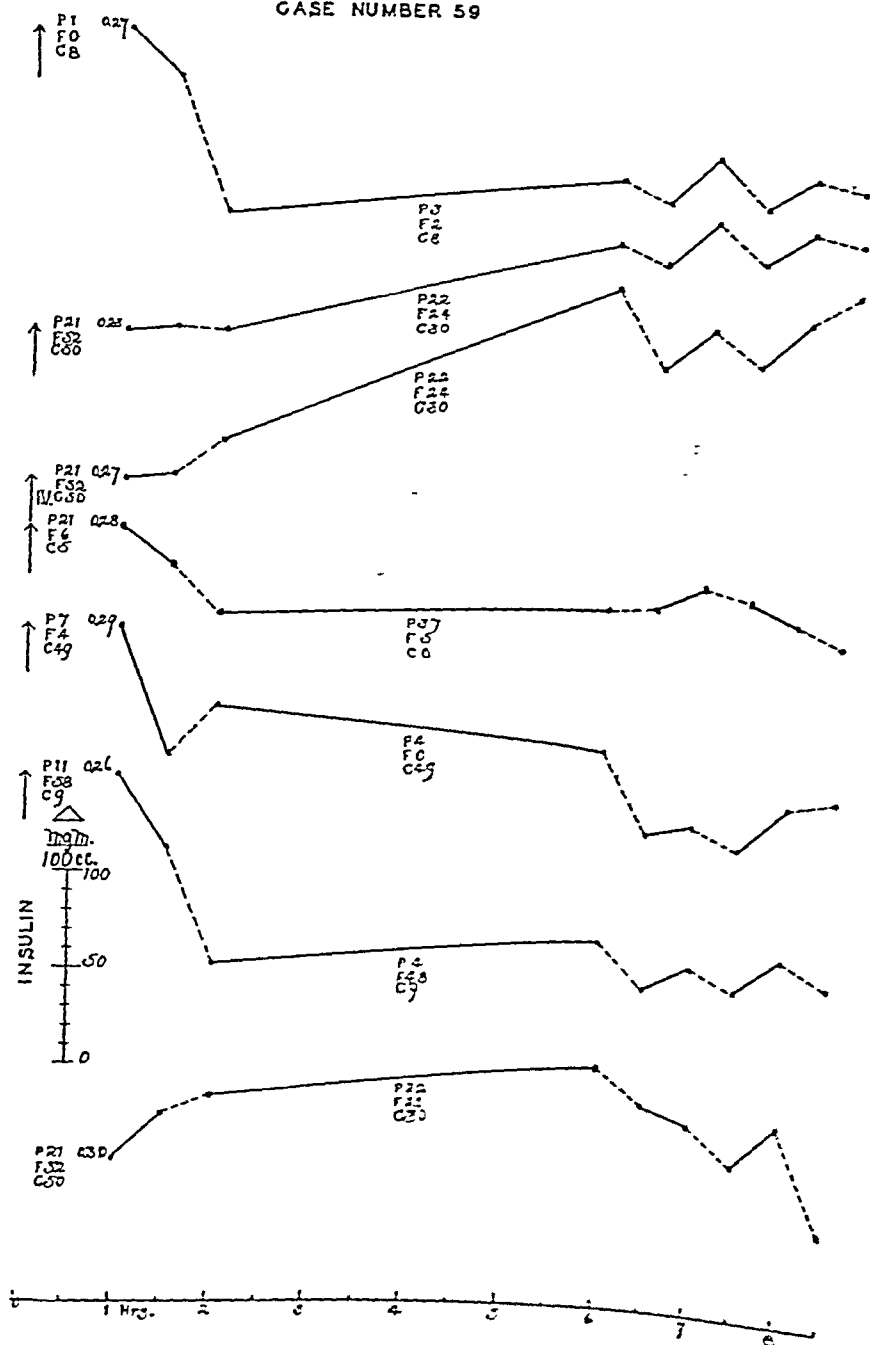
FIG. 7. SHOWING BLOOD SUGAR CURVES OF ALL DAY EXPERIMENTS ON PATIENT 59

———— = rest   — — — — = exercise.
⌇ indicates 5 units insulin.

has been called the "muscle glycogen vacuum." Exercise probably excites an increased availability of both adrenalin and insulin, thereby supplying more glucose to the blood and converting it in turn to the use of the exercising muscles.

Excessive exercise in the normal subject may be accompanied by a marked hypoglycemia. This is usually attributed to a depletion of the stores of glycogen in the body. In the diabetic the glycogen of both muscles and liver is usually lower than normal. With this low glycogen content of the tissues exhaustion of the stores might be expected to occur more readily and this is a plausible explanation of the tendency to hypoglycemia after exercise in the mild diabetics who are still able to withdraw glucose from the blood during exercise.

When the diabetes is so mild that a normal blood sugar follows a 16-hour fast, the blood sugar curve after exercise is apparently normal. There is only a slight change in blood sugar with exercise and that change is usually downward. It appears that the normal protection against marked hypoglycemia is still effective in these patients.

With a little less mild diabetes, indicated by a slightly higher fasting blood sugar, a greater drop occurs after exercise. The blood sugar seems to show the greatest decrease when it starts from approximately 175 mgm. per 100 ml. of blood. It may be presumed that in such cases the stimulus of exercise makes demands upon the blood sugar which the mechanisms or the reserves of the body can not quite adequately support.

As the ability of the body to use carbohydrate becomes still further impaired a different factor predominates. The stimulus of exercise seems to lead to less withdrawal of blood sugar. Presumably this is due to deficient insulin. Little or no change occurs in the blood sugar level during exercise. This behavior was usually observed with a fasting blood sugar between 200 and 300 mgm.

When the diabetes is of sufficient degree so that the fasting blood sugar is above 300 mgm. the reduction in the ability to withdraw sugar from the blood was, in almost all the severe diabetics studied, so marked that in these individuals the blood sugar actually rose during a ½ hour period of exercise as is shown in Figure 1, third column. After only 5 minutes of exercise there was a definite decrease in the blood sugar in some of the severe diabetics, but after a further period of exercise the increase occurred. It would appear, therefore, that in some patients there was a sharply limited capacity for withdrawing blood sugar which was exhausted during the first few minutes of exercise. If the decreases in blood sugar following exercise are dependent on available insulin then the food intake in the day in Cases 59 and 55 appears to have increased the availability of the patients' insulin; for no insulin had been taken by these patients for almost 24 hours (see Figures 6 and 7, last curves).

Comparisons between the effects of exercise on blood sugar in the mild and in the severe diabetic suggest that, in the former, the difficulty is one of inadequate or inadequately available glycogen reserves and, in the latter, a more or less complete inability to convert glucose in the blood to the needs of the tissues.

CONCLUSIONS

A standard form of exercise has been used with 61 diabetic patients with blood sugar determinations made before and after the period of exercise.

The effect of exercise of the degree and character employed in these experiments, after 16 hours without food or insulin, varied with the severity of the diabetes as indicated by the fasting blood sugar level.

With increase in the fasting blood sugar level from a normal value to about 175 mgm. per 100 ml. of blood the effect of a half hour of standardized exercise induced a progressively more marked *lowering* of the blood sugar.

With further increase in the fasting blood sugar level from 175 mgm. to above 300 mgm. the effect of a half hour of standardized exercise induced a progressively more marked *elevation* of the blood sugar.

In the severe diabetic the intravenous injection of 0.1 unit of insulin, which given before a period of rest was without marked effect on the blood sugar, caused, when given immediately before a period of exercise, a recognizable drop in blood sugar. Five times this dose given intravenously a half hour before beginning the exercise failed to prevent in the severe diabetic this rise associated with the exercise.

The effect of 0.5 to 5 units of insulin subcutaneously was not influenced by rest or exercise.

Recent intake of food led under certain conditions to a decrease in blood sugar during exercise in two diabetics, who, fasting, exhibited a rise of blood sugar during exercise.

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THE LACTIC ACID AND GLUTATHIONE CONTENT OF THE BLOOD OF SCHIZOPHRENIC PATIENTS [1]

By JOSEPH M. LOONEY and HAZEL M. CHILDS

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The theory that schizophrenia is due to a deficient oxidation of the body tissues particularly in the hypothalamic region of the brain has been the starting point for a number of investigations. In support of this theory it has been demonstrated (1) that the rate of oxygen consumption of schizophrenic patients is significantly lower than that of normal subjects. This decreased intake of oxygen is not infrequently (2) accompanied by a lowering of the venous oxygen content of the blood. McFarland (3) has shown that when normal individuals are subjected to a lowered oxygen tension for a short time they tend to react in a manner very similar to schizophrenic patients.

In view of these facts it was felt that a study of the lactic acid content and also of the oxidative enzymes of the blood might shed further light on the problem. Even though sufficient oxygen were made available, a deficiency in amount of glutathione which is a general cell catalyst that facilitates oxidation-reduction reactions might interfere with the utilization of this oxygen by the tissues and thus lead to an accumulation of lactic acid.

The investigation was carried out on a group of 37 schizophrenic patients and 18 normal subjects. The patients were all males, between the ages of 20 and 45, free from all evidences of ordinary organic disease such as could be detected by a careful physical examination and a thorough study of the blood and urine by exhaustive laboratory procedures. All cases in which the diagnosis of schizophrenia was not agreed to by the diagnostic staff of three or more psychiatrists, several internists, and two psychologists—both of the latter groups being well versed in practical psychiatry—were excluded from the study. The normal subjects consisted of male physicians, attendants, medical students, and employees of the hospital. These were all presumably healthy individuals and free from acute infections at the time of the test.

The blood samples were all taken from the median basilic vein, with-

out stasis, which was avoided by waiting for at least one minute after the removal of the tourniquet used to facilitate entrance into the vein.

The subjects reported for the test in the morning, in a fasting condition, and then reclined quietly in bed for 30 minutes before the blood samples were taken.

The amount of total and reduced glutathione was determined by the method of Woodward and Fry (4) on sulfosalicylic acid filtrates; the lactic acid analyses were made by the method of Friedemann and Kendall (5). The gas analyses were made by the manometric method of Van Slyke. All analyses were made in duplicate from blood collected and stored in a capped syringe in the manner described by Looney and Childs (6).

An analysis of the technical errors involved in the determination of lactic acid and also of glutathione indicated that these were entirely negligible, as about 80 per cent of the duplicate determinations on the same blood sample differed by only 0.5 mgm. and about 90 per cent of the double runs varied less than 1.0 mgm. Some difficulty was encountered in the lactic acid determinations owing to the fact that oxidizable materials in the laboratory air gave excessively high blanks, but this was readily overcome by the insertion into the system of two wash bottles, the first containing bisulfite solution and the second permanganate solution, so that all air carried through the apparatus had first to pass through these wash bottles. Since the introduction of this modification the recovery of lactic acid, added in the form of lithium lactate, has varied from 99 per cent when 20 mgm. were added to 96 per cent when 10 mgm. were added.

The distribution constants for the various constituents studied are given in Table I for the venous blood of the patients and of the control subjects. There is a significant difference between the mean value of 59.30 ± 0.47 volumes per cent for the carbon dioxide content of the blood of the patients, and that of 54.12 ± 0.86 volumes per cent for the control subjects. The difference for the venous oxygen content is 1.38 volumes per cent, the mean for the patients being 8.74 ± 0.38 volumes per cent and that for the normal subjects being 10.12 ± 0.67 volumes per cent. The sample taken from the patients' group is not representative of the schizophrenic population as a whole in respect to blood gases since patients showing low venous oxygen values on previous studies were purposely chosen for this investigation.

The mean value for the lactic acid content of the venous blood of the patients was 14.27 ± 0.72 mgm. per cent, while that for the control subjects was 10.28 ± 0.57 mgm. per cent. The distributions for both groups are shown in Figure 1. The difference, 4.00 ± 0.92 mgm. per cent, exceeds its standard error by more than four times and is therefore statistically highly significant. With a larger sample the difference might be somewhat smaller than that found in this study because many of the high values seemed to occur among the early determinations. But even

TABLE I

Constants of distributions pertaining to venous gases, lactic acid, etc. of schizophrenic patients and normal controls

		Num-ber	Mini-mum	Maxi-mum	Mean and ϵ_m	S.D. and $\epsilon_{S.D.}$
CO_2, *volumes per cent*	Patients	51	53.25	73.09	59.30±0.47	3.33±0.333
	Controls	17	49.72	63.52	54.12±0.86	3.44±0.61
O_2, *volumes per cent*	Patients	51	4.21	15.69	8.74±0.38	2.69±0.269
	Controls	17	6.78	17.53	10.12±0.67	2.69±0.48
Reduced glutathione, *mgm. per cent*	Patients	53	25.00	45.40	34.99±0.68	4.90±0.45
	Controls	18	31.30	47.90	38.23±1.20	4.96±0.85
Total glutathione, *mgm. per cent*	Patients	49	27.90	50.30	39.12±0.71	4.94±0.5
	Controls	18	33.80	51.80	41.91±1.25	5.14±0.88
Lactic acid, *mgm. per cent*	Patients	57	6.70	38.40	14.27±0.72	5.35±0.51
	Controls	18	6.10	13.90	10.28±0.57	2.33±0.40

when these values are excluded the difference still remains significant. The standard deviation for the patients of 5.35 ± 0.51 mgm. per cent is more than twice as great as the value obtained for the normal subjects of 2.33 ± 0.40 mgm. per cent. The lactic acid content of the blood of schizophrenic patients is, therefore, not only higher than that of normal subjects but is also subject to much greater variation among individuals.

The mean of reduced glutathione for the patients was 34.99 ± 0.68 mgm. per cent, while that for the normal subjects was 38.23 ± 1.20 mgm. per cent. The difference is 3.24 mgm. per cent, while the standard error of this difference is 1.38 mgm., so that the difference exceeds its standard error only 2.3 times, which indicates that a similar difference could occur by chance about once in forty trials, and as most statisticians require that the possibility that the change is due to chance be no greater than one to fifty, we cannot consider that we have demonstrated a statistically significant lowering in the reduced glutathione of the patients. As the decreased glutathione might, in part at least, account for high lactic acid values, the difference may be regarded as of some physiological importance.

The mean values for total glutathione were 39.12 ± 0.71 mgm. per cent for the patients, and 41.91 ± 1.25 mgm. per cent for the normal subjects. The difference of 2.79 mgm. per cent is not statistically significant.

The values for the constituents in the arterial blood are given in Table II. It will be noted that the amounts of total glutathione and reduced

FIG. 1. DISTRIBUTION OF VENOUS LACTIC ACID VALUES IN NORMAL SUBJECTS AND PATIENTS

glutathione are practically the same as in the venous blood, the slight difference shown being only about one-half the standard error. The lactic acid content has dropped to 11.81 ± 0.61 mgm. per cent. The oxygen content and the carbon dioxide content of the arterial blood agree with the values obtained in our previous study.

TABLE II

Constants of distributions pertaining to arterial gases, lactic acid, etc. of schizophrenic patients

	Number	Minimum	Maximum	Mean and σ_m	S.D. and $\sigma_{S.D.}$
CO_2, *volumes per cent*	32	42.13	63.33	49.73 ± 0.76	4.26 ± 0.54
O_2, *volumes per cent*............	32	8.91	22.35	19.11 ± 0.48	2.68 ± 0.34
Reduced glutathione, *mgm. per cent*.....................	30	22.00	43.00	35.4 ± 0.88	4.72 ± 0.62
Total glutathione, *mgm. per cent*..	30	26.00	50.60	38.66 ± 0.82	4.43 ± 0.58
Lactic acid, *mgm. per cent*.......	30	5.60	27.60	11.81 ± 0.61	3.39 ± 0.43

A definite correlation was found between the venous and arterial lactic acid, a coefficient of 0.81 being obtained. For the correlation between venous and arterial reduced glutathione a coefficient of 0.88 was found.

As a first approximation to the solution of the problem whether the high lactic acid values in the patients were due to lack of oxygen. the coefficients of correlation were computed for the various interrelationships of the variables studied in the venous blood. No correlation was found between any of the various functions, the highest coefficient, —0.15. being that for the relationship between lactic acid and carbon dioxide. None of the other coefficients exceeded 0.08. The lack of correlation between the level of oxygen and that of lactic acid was somewhat surprising. as the cases studied had been picked on the basis of low venous oxygen values obtained in previous examinations. *Since the lactic acid level was independent of the amount of oxygen supplied to the tissues, the high value must be ascribed to some local factor which interferes with oxidation*. If a relationship had been found between the lactic acid values and the oxygen we should not be justified in stating that the high values were characteristic of schizophrenia. The failure to find such a relationship indicates that the method of choosing the cases for study had no influence on the results so that the sample may be considered as a truly random one in respect to lactic acid.

The work of Margaria, Edwards, and Dill (7) has shown that the concentration of lactic acid in venous blood is proportional to the amount of lactic acid in the tissues. The high values of lactate noted by us, therefore, indicate an accumulation of lactic acid in the tissues. Lactic acid is an intermediate product in the oxidation of glucose. Since the abnormality cannot be ascribed to inadequate oxygen supply it must be due to something that interferes with the oxidative reaction. This disturbing factor might be either a lack of oxidative catalysts, or the presence of some toxic material which interferes with oxidation, or both conditions together. Part of the increase in lactic acid may well be due to a lack of reduced glutathione even though the decrease in this substance is not great enough so that the difference can be held to be statistically significant. Certain evidence that we have accumulated leads us to believe that the latter factor is also operative: LeClair and Looney (8) have shown that the rate of respiration of the luminescent bacterium. *Vibrio phosphorescens*, is less in the presence of serum from schizophrenic patients than it is when an equal amount of normal serum is added to the suspension of bacteria.

<div align="center">CONCLUSION</div>

Lactic acid is not removed from the tissues of schizophrenic patients in a basal state as readily as it is from normal subjects. This failure may be due in part to a decrease in the content of reduced glutathione.

FIG. 1. DISTRIBUTION OF VENOUS LACTIC ACID VALUES IN NORMAL SUBJECTS
AND PATIENTS

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THE SIGNIFICANCE OF CONCENTRATION AND DILUTION TESTS IN BRIGHT'S DISEASE

By ALF S. ALVING and DONALD D. VAN SLYKE

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The first quantitative evidence that nephritis diminishes the ability to excrete urine of either high concentration or high dilution was apparently furnished by Korányi (1899) and his collaborators, Kövesi and Roth-Schulz (1900), by means of the freezing point method. Regular application to studies of renal function, however, appears to have first begun in Volhard's (1918) clinic, where the "concentration and dilution" test has been in use since about 1908. In this test the renal function is estimated from the ability to excrete a urine of large volume and low specific gravity after drinking 1.5 liter of water (dilution test), and urine of high specific gravity after a subsequent period in which no fluid is drunk (concentration test). Modifications of these tests or their combination have been made by various authors. They have been reviewed by Pratt (1926) (with his own observations on 58 patients), by Mosenthal (1930), by Lashmet and Newburgh (1930), and with especial completeness by Volhard and Becher (1929). The simplicity of these tests, and their consequent wide application, render it desirable to define as exactly as possible the meaning of their results.

In order to obtain information for this purpose we have made graphic statistical comparisons of results of concentration tests with results of the urea clearance test, the clinical significance of which has previously been established (Van Slyke, Stillman, Möller, *et al.* (1930) [1]). We have furthermore carried out prolonged observations with concentration tests and urea clearance combined on individual patients with the different types of Bright's disease, so that the prognostic significance of the tests could be deduced from the clinical outcome.

While our studies have been in progress, other data comparing results of concentration and dilution tests with results of the urea clearance test have been published by Ong (1932) from Snapper's clinic, and by Bruger and Mosenthal (1932).

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[1] The terms, "standard blood urea clearance" and "maximum blood urea clearance" used in expressing the urea excreting efficiency of the kidneys, have been defined by Möller, McIntosh and Van Slyke (1928).

METHODS

Two forms of the concentration test (Addis and Shevky (1922); Lashmet and Newburgh (1930)) and one of the combined concentration and dilution test (Lundsgaard (1920)) have been used in this clinic for comparison with the blood urea clearance. In addition to our results with these tests we have charted the data of Ong (1932) and of Bruger and Mosenthal (1932). The different concentration and dilution tests, as used by the writers and the above authors, were applied as follows.

Concentration tests

The concentration test of Addis and Shevky (1922). The subject abstains from fluids of all sorts from after breakfast on one day until he rises from bed on the morning of the following day. During the last 12 hours of the dry period (from 8 p.m. to 8 a.m.) the urine is collected, and the specific gravity of this specimen is measured. The Addis-Shevky test prescribes no preparatory period on a special diet.

In applying this method to albuminous urines we have subtracted from the observed specific gravity a correction for the effect of the protein, in the manner described below for the Lashmet-Newburgh test. The gravities given in our charts are the corrected values. The urinometer used has been found accurate to within 0.001.

Addis and Shevky have found that in normal individuals, previously on ordinary diets, the average specific gravity of urine obtained as above described was 1.032, that 95 per cent of normal subjects on ordinary diets showed values of 1.028 or above, and 100 per cent above 1.026.

The concentration test of Lashmet and Newburgh (1930).[2] The subject remains in bed four days, during the first three of which he is on a special diet. The diet consists of 45 grams of protein, 106 grams of fat, and 180 grams of carbohydrate. The sodium chloride intake is from 2 to 3 grams daily, and the fluid 1500 cc. At 6 p.m. on the third day, after the patient has had supper, all intake, both food and fluid, is stopped until noon the following day. All urine is discarded except the specimen obtained from 10 a.m. until 12 noon on the fourth day. The specific gravity of this two-hour urine specimen is determined. We did this by means of small weighing bottles as described by Moore and Van Slyke (1930). When protein was present in significant amounts the observed specific gravity was corrected by subtraction of the increment in gravity due to the protein, as was done by Lashmet and Newburgh (1930).[3] Their more recent (1932) correction curve corresponds to the equation:

$$\text{Sp. Gr. correction} = 0.003 \times (\text{per cent protein}).$$

Lashmet and Newburgh found that all normal subjects concentrated the urine to 1.026 or above on this regime.

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[2] Since the completion of this paper Lashmet and Newburgh (1932) have altered their test, omitting the three-day preparatory period, but increasing the period of desiccation to 24 hours.

[3] If the specific gravity of the protein in the urine is not taken into account, there is wider scattering of points in Figure 1.

Combined concentration and dilution tests

Lundsgaard's modification of the Volhard tests. Christen Lundsgaard (1920, unpublished) slightly modified for use in this hospital the Volhard concentration and dilution test. *Dilution.* The subject receives no water after midnight. He voids at 7 a.m., then drinks 1000 cc. of water and voids each hour till 11 a.m. *Concentration.* The concentration test is done on a different day. No water is taken after preceding midnight. A dry meal, consisting of 65 grams toast, 15 grams butter, 100 grams scrambled eggs, 25 grams cream, and 15 to 20 grams jam or jelly, is given at 7:30, and repeated at 10:00 and 11:40 a.m. Urine is voided every 2 hours from 7 a.m. to 3 p.m. The specific gravity in normal subjects rises above 1.025, and usually as high as 1.030.

Rosenberg's (1927) modification of the Volhard tests. Rosenberg added precautions to make certain that the body was in a normal and steady state of hydration. For 2 days before either the concentration or dilution test, which were done on different days, the subject was put on a controlled diet, without unusual variations in salt, and with 1500 cc. of water. The weight of the subject was taken during the days before and after the test in order to detect instability in the water balance, due to the invalidating factors mentioned below, or others. For the rest, the technique was essentially that of Volhard, except that in the dilution test only 1000 instead of 1500 cc. of water were taken. The urine was collected in half-hour periods for 4 hours after drinking the water. During the next 24 to 48 hours a dry diet was taken, and the urine was collected every hour or two, till its specific gravity reached a maximum. Rosenberg's modification was used by Ong (1932), whose results are discussed later. To judge from comparison of Figure 1 and Figure 4-A the preliminary regime had little influence in making the results more uniform.

Mosenthal's (1915, 1918) test based on spontaneous variations in the specific gravity of the day urine and on the volume of the night urine. This is a modification of the Hedinger and Schlayer test (1914). The subject is allowed to follow his own desires concerning intake of food and fluids. Restriction of diet was found by Mosenthal (1918) to be unnecessary. The test depends on the facts that, with ordinary fluid intake, even at a constant rate per hour, the hourly urine volume and specific gravity vary markedly during the day in normal subjects, and that the extent of the variation diminishes in nephritis. The urine is collected in 2-hour periods from 8 a.m. to 8 p.m., and in a 12-hour period from 8 p.m. to 8 a.m. the next morning. The specific gravities of the 2-hour specimens are measured, and the volume of the 12-hour night specimen. The normal values given in Mosenthal's most recent paper (Bruger and Mosenthal (1932)) are a specific gravity of 1.020 or above in one or more of the 2-hour specimens, a specific gravity difference of not less than 0.009 between the highest and lowest specimen, and a volume less than 725 cc. for the 12-hour night urine.

The urine protein was measured by the method of Shevky and Stafford (1923) as slightly modified by MacKay (Peters and Van Slyke, 1932, II, p. 682).

The methods used in determining and calculating the standard and maximum urea clearances have been described by Möller, McIntosh and Van Slyke (1928). The clearance has been recorded in percentages of

the mean normal values, which are 54 cc. per minute for the "standard" clearance, determined with low urine volumes, and 75 cc. for the "maximum" clearance, determined with high urine volumes, with due correction for body size in cases much above or below average adult stature (McIntosh, Möller, and Van Slyke (1928)).

Urea in the blood and urine was determined by the gasometric urease method (Van Slyke (1927)).

Urine total base was determined gasometrically as described for blood by Van Slyke, Hiller, and Berthelsen (1927). The method described by
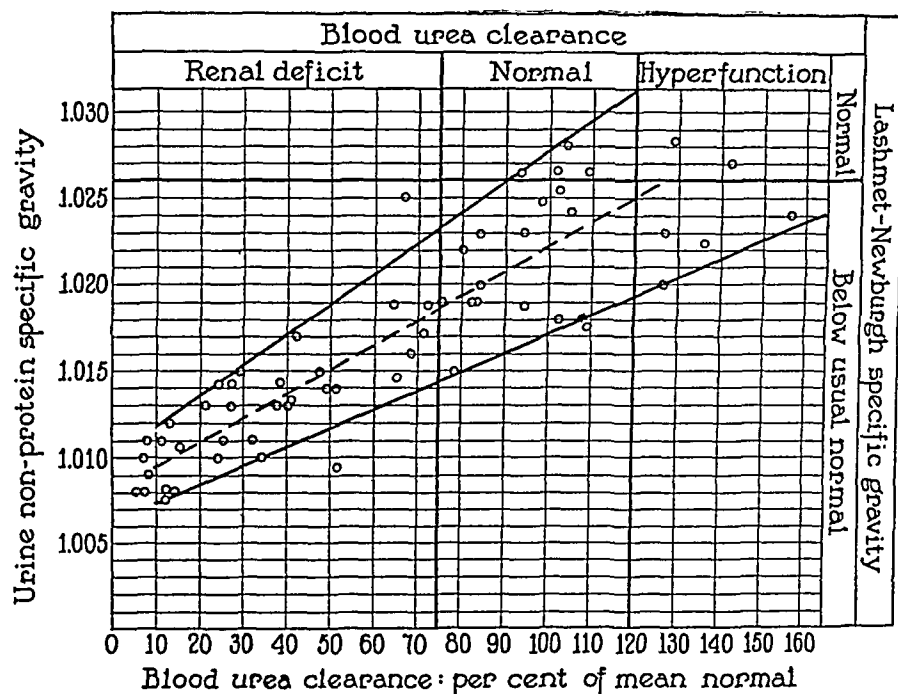
FIG. 1. LASHMET-NEWBURGH CONCENTRATION TEST

Each circle represents results of the concentration test and the urea clearance with one subject. The gravities are corrected for the effect of urine proteins. All the subjects were nephritic patients. Hence the presence of low specific gravities in subjects with normal urea clearances.

these authors for serum was applied to urine without change, except in the size of samples used. The total base in urine of different concentrations varies so greatly that it is desirable to vary the samples so that each will contain the amount of base in 1 cc. of a 100 to 200 milli-equivalent solution. The size of sample required can be estimated from the nonprotein specific gravity of the urine, as indicated by Figure 14.

RESULTS OF CONCENTRATION TESTS

Statistical comparison with the urea clearance test

The Lashmet-Newburgh test was compared with the clearance in 39 subjects, and the results are shown in the dot diagram of Figure 1. The

Addis-Shevky test was performed on the same subjects, and in addition data were available for a number of other patients who had previously been put on the Addis 20-hour dry regime in order to obtain concentrated urine for count of the formed elements (Addis (1925)). The results of the Addis-Shevky test are presented in Figure 2. The results with Lundsgaard's modification of the Volhard test are given in Figure 3, the data of Ong with the Rosenberg modification of Volhard's test are reproduced in Figure 4, and Bruger and Mosenthal's results with the Mosenthal test in Figure 5.

The specific gravity values in the Addis-Shevky and Lashmet-Newburgh tests (Figures 1 and 2), were corrected for the protein present.
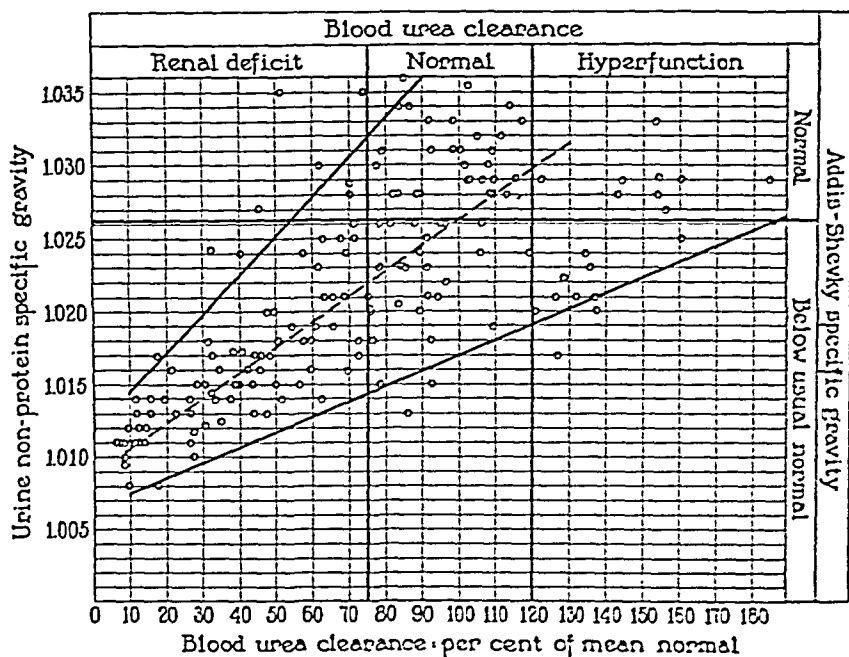
FIG. 2.   ADDIS-SHEVKY CONCENTRATION TEST

Each circle represents results with one subject. The gravities are corrected for the effect of urine proteins. All the subjects were nephritic patients. Hence the presence of low specific gravities in subjects with normal urea clearances.

The data from previous authors, presented in Figures 3–A, 4–A, and 5–A. lack this correction, so that the urine specific gravities shown in cases with heavy albuminuria are higher than they would be if corrected. It is probable therefore that in these figures, especially Figure 4–A, some of the cases which show normal specific gravity with subnormal urea clearance, would show subnormal urine gravities and better correlation with the clearance, if the protein correction were applied.
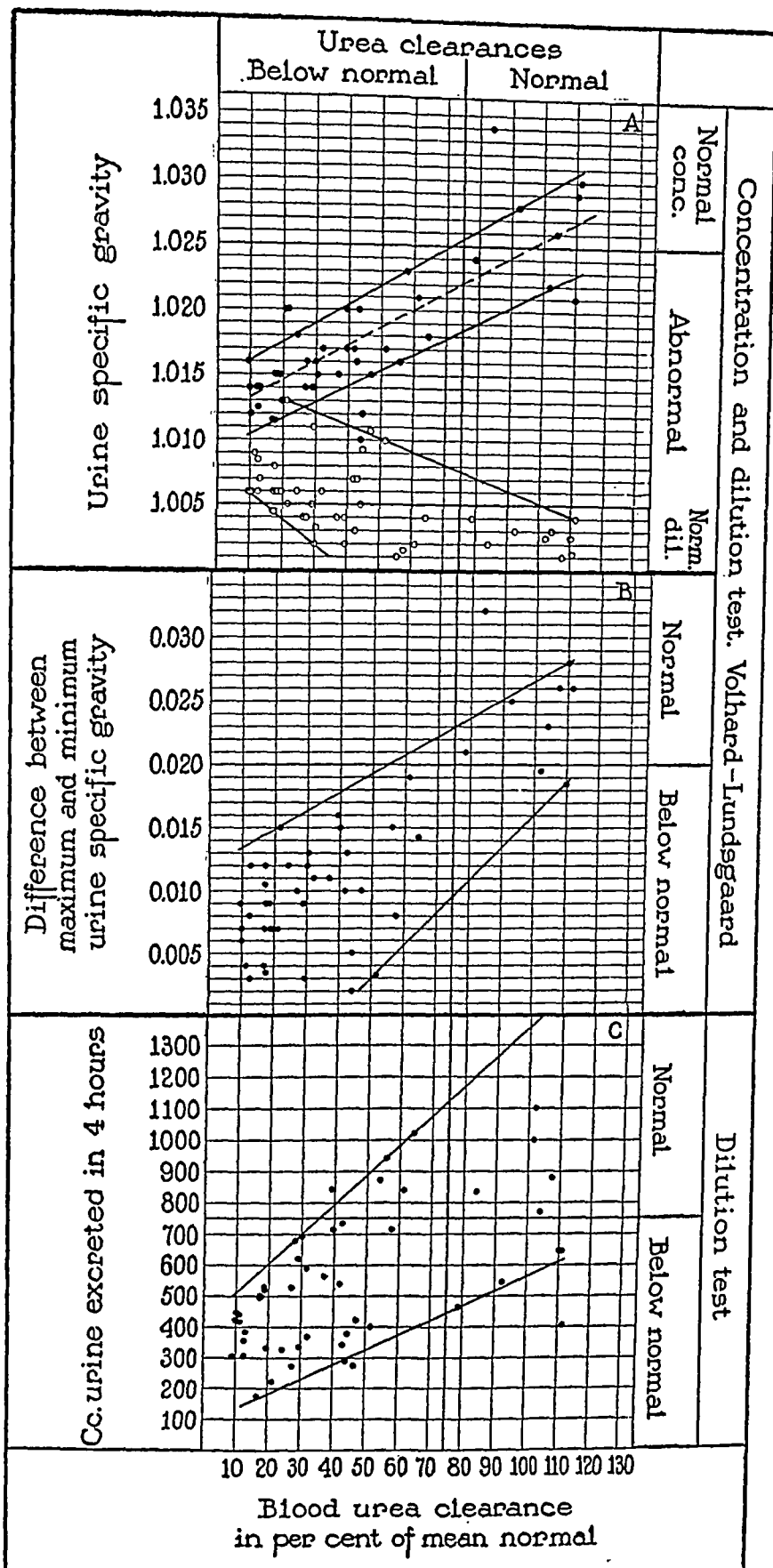
FIG. 3. VOLHARD-LUNDSGAARD CONCENTRATION AND DILUTION TEST

Each circle represents the blood urea clearance and the result of the concentration or dilution test with one subject. In chart A solid circles represent maximum specific gravities obtained in the concentration test. Hollow circles represent minimum specific gravities obtained in the dilution test. The gravities are not corrected for the effect of urine protein concentration. Data of Lundsgaard (1920) and successors. All the subjects were nephritic patients.
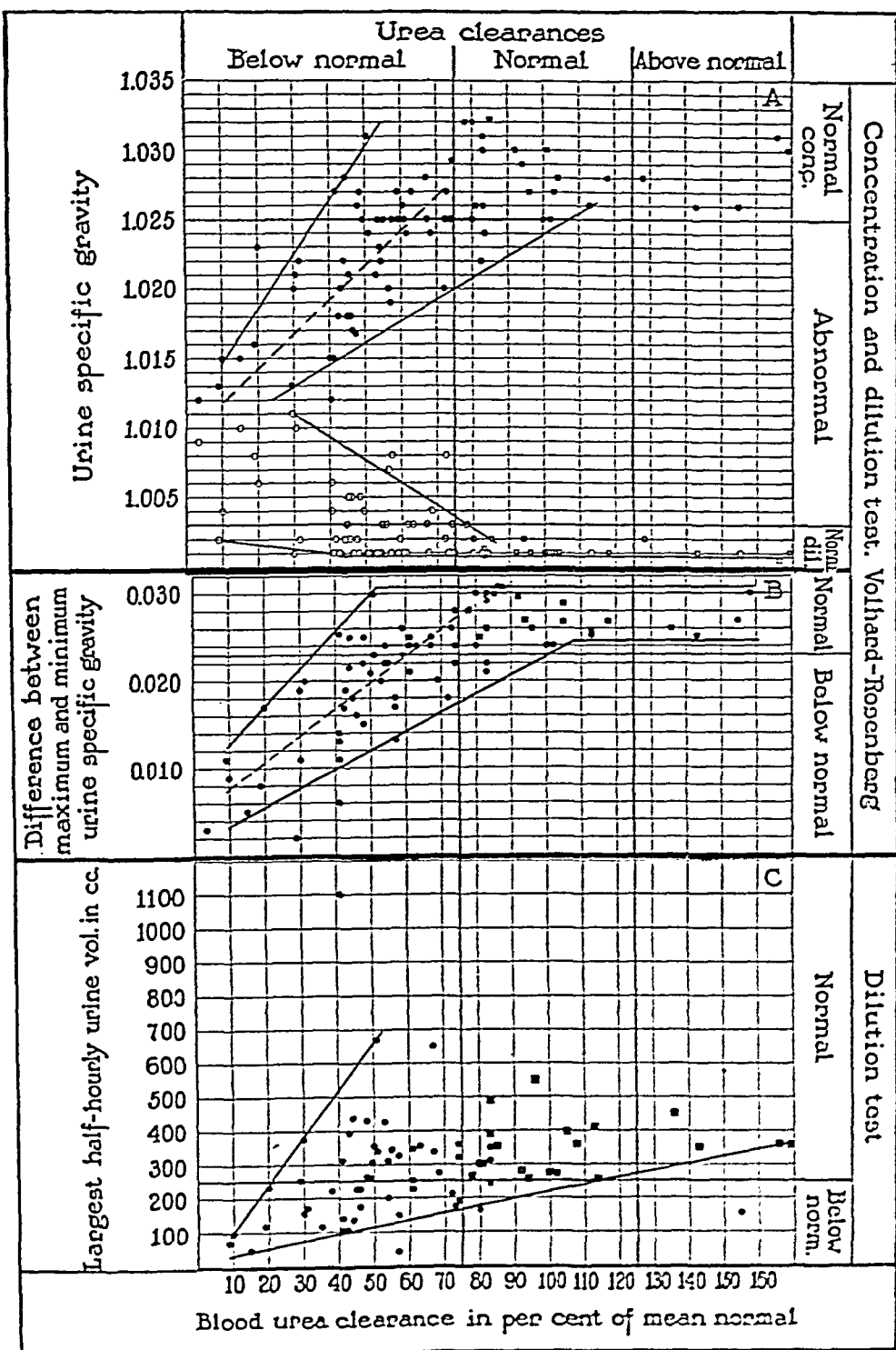
Fig. 4. Ong's Results with Volhard-Rosenberg Concentration and Dilution Test

Maximum specific gravities, obtained in the concentration test, are represented by solid circles or squares: minimum gravities, obtained in the dilution test, are represented by hollow circles and squares. The gravities are not corrected for the effect of urine protein concentration. Circles represent results in nephritic subjects. Squares represent results in normal subjects.
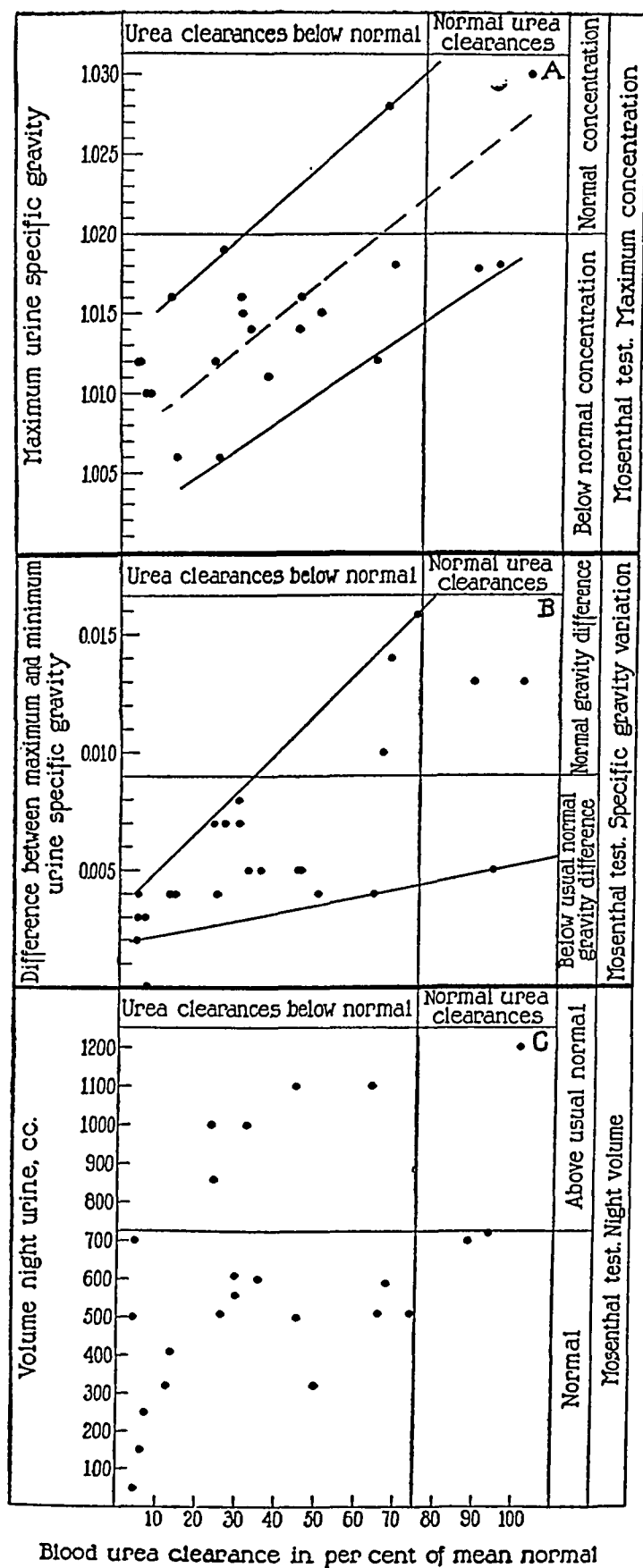
Fig. 5. Results of Bruger and Mosenthal (1932) with the Mosenthal (1918) Spontaneous Concentration and Dilution Test

All the subjects were nephritic patients.

It is obvious from the dot diagrams of Figures 1, 2, 3–A, 4–A, and 5–A, that *nephritic patients with normal urea clearances frequently show low specific gravities by the concentration tests.* (Normal clearances are encountered with some frequency, in the presence of obvious renal abnormality, in the following conditions: in mild acute nephritis, in the recovery stage of acute nephritis, and in some cases of nephrosis (Van Slyke, Stillman, Möller, *et al.* (1930)).)

In *cases with less than normal urea clearances,* the charts show correlation between the fall in clearance and the fall in specific gravity.

In so far as the different tests compare, the relatively narrow area enclosing all but 2 of the points on Figure 1 appears to indicate that the Lashmet-Newburgh concentration test agrees, somewhat more consistently than the other concentration tests, with the urea clearance. Somewhat greater consistency might be expected of the Lashmet-Newburgh test because of the more complete preparation, with the 3-day preliminary regime. However, the 2-day preliminary regime of the Rosenberg test (Figure 4–A) appears to give, with the urea clearance, no obviously closer correlation than the 12-hour preliminary regimes of the Addis-Shevky (Figure 1) or Lundsgaard (Figure 3–A) tests. Mosenthal's test is distinguished from the others in that it involves no preliminary desiccating period at all, and merely measures the maximum specific gravity that occurs spontaneously during a day on unrestricted solid and fluid intakes. The chief effect is that the general level of the specific gravities obtained in the Mosenthal test is lower than in the others, the normal low limit being at 1.020 instead of 1.025 to 1.026, while the trend of the pathological values is also lower in the Mosenthal test. Aside from this difference, and some apparent loss in sensitivity entailed, the Mosenthal test appears to give results of the same significance as the concentration tests done with regimes of prescribed solid and fluid intake.

In general, Figures 1 to 5 indicate that in nephritic cases with low urea clearance, all the concentration tests yield urine specific gravities tending to fall with diminishing urea clearance until the clearance has fallen to 20 or 30 per cent of normal. The broken slanting line in each chart indicates approximately the mean relationship between clearance and urine specific gravity, while the area enclosed within the two solid slanting lines indicates the extremes of variation that may ordinarily be expected. Thus, in Figure 2, it is apparent that a urine specific gravity of 1.016 in the Addis-Shevky test corresponds on the average to a urea clearance 40 per cent of mean normal. but may coincide with urea clearances anywhere from 16 to 90 per cent of mean normal. That the clearance, in a case with a given urine specific gravity, may occasionally be outside of even such a wide range. is indicated by the presence of a few dots outside the area between the two solid lines. It is evident that a given subnormal

specific gravity may accompany either a very low urea clearance, or one within the normal range.

More specific information concerning the significance of the concentration tests is yielded by continual observation of individual cases through the different stages of renal disease. The results of such observations will next be considered.

Acute hemorrhagic nephritis [4]

Acute cases with both tests normal throughout. Several cases of transitory acute nephritis with normal urea clearances have been reported by Van Slyke, Stillman, *et al.* (1930). In Figure 6 are charts [5] of three cases which throughout the period of observation gave normal results, not only by the urea clearance, but also by the concentration test. The hematuria, proteinuria, and initial edema, however, leave no doubt that each patient passed through an attack of acute nephritis.

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[4] The nomenclature used to indicate the different types of nephritis is that outlined by Van Slyke, Stillman, Möller, *et al.* (1930), as developed from Volhard and Fahr, and Addis.

[5] *Explanation of Case Charts.*

For renal function, hemoglobin in blood, hematuria, plasma proteins, and blood pressure the mean normal is drawn as a base line; the shaded areas between the base line and the points representing observations indicate the degree of deviation above or below the average normal. When the shaded area extends downward from the base line the observed value is below the normal average, and *vice versa.* Gross hematuria is plotted as columns extending above the 100 million mark.

The brackets at the left of the scales for urea clearance, urine concentration and hematuria indicate the range of normal variability. The normal base line for hemoglobin values varies with the patient's age and sex.

The scales for urine protein and diet are obvious.

The black areas representing edema have the following significance.

Height of black area in quarters of total space   Edema

Height of black area in quarters of total space	Edema
1	Trace
2	Moderate pitting
3	Marked pitting
4	General edema with ascites

Under fundus changes we chart constriction of the arterioles, arteriolar sclerosis, exudate, retinal hemorrhages, and papilledema. The degree of development of each change is indicated by the proportion of the indicated area on the chart which is blackened; a minimal change is indicated by blackening of $\frac{1}{4}$ of the area, and a maximal one by blackening of the entire area.

The vertical spaces which are left unshaded for the entire length of a chart indicate periods during which no observations were made, usually because the patient was out of the hospital.

At the bottom of each chart the number at the left nearest "months" indicates the number of months the disease was noted before the patient entered the hospital. The other numbers in the bottom row indicate months after first admission.
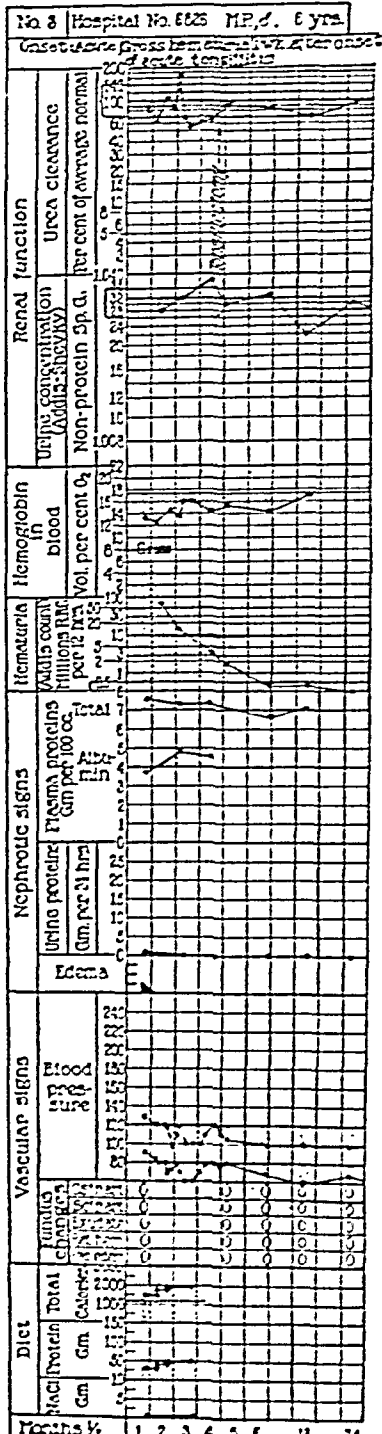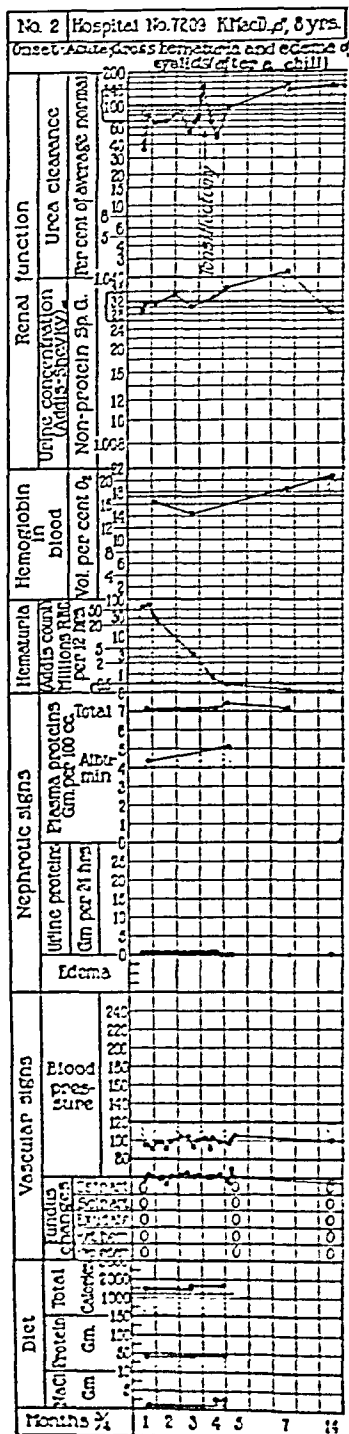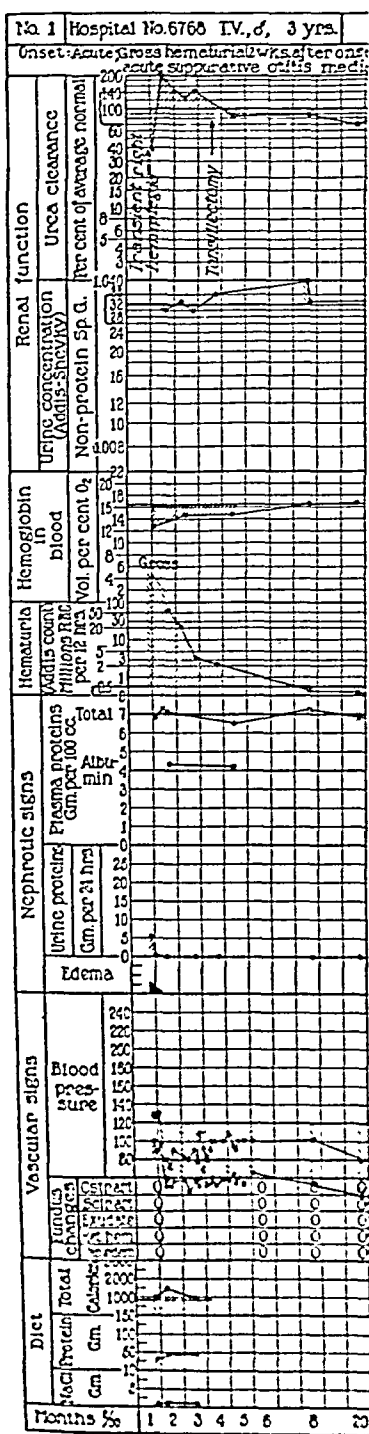
FIG. 6. THREE CASES OF TRANSITORY ACUTE HEMORRHAGIC NEPHRITIS WITHOUT FUNCTIONAL EFFECT ON EITHER UREA CLEARANCE OR CONCENTRATION TEST

See footnote 5, page 978, for explanation of case charts.

Acute cases in which both tests are depressed, with subsequent return of the clearance to normal, while low specific gravity persists for some time longer. In patients recovering from acute nephritis the urea clearance frequently rises to normal level sooner than does the urine specific gravity. This behavior is exemplified by the two cases in Figure 7. Three months after onset of acute nephritis in Case 4 the urea clearance had risen to 80 per cent of average normal, a value well within normal range, while the urine specific gravity in both the Addis-Shevky and the Lashmet-Newburgh tests had risen from 1.010 to 1.012 only as far as 1.019–1.020. In Case 5 the time lag of the concentration test was still more striking. Three months after onset the clearance had returned to normal, while the urine specific gravity by the Addis test still remained at 1.011 to 1.012, a value frequently found in uremia. Later the urine specific gravities in both cases gradually rose till they were within normal limits.

Usually, as in these two cases, if the specific gravity test in an improving patient fails to reach normal as soon as the urea clearance, it attains normal level a few weeks or months later. In exceptional cases, however, the period during which subnormal concentration tests persist despite normal urea clearance has lasted for several years, as exemplified by Case 7 in Figure 8.

Advantages of observing both tests in acute nephritis. The question arises, whether a low concentration test, persisting after recovery of normal urea clearance and subjective well-being, is significant. Our observations lead us to believe that it is, and that the continuance of low concentration tests indicates the persistence of some renal damage. In most cases during the period when the concentration test lags behind the clearance in regaining normal level, the persistance of hematuria, proteinuria, or other signs indicates that the kidneys are not yet normal (Cases 4 and 11), though occasionally (Case 5) low concentrating ability may persist for some time after all other signs of renal disease have disappeared. Sometimes an exacerbation adds further unwelcome proof that healing is not yet final (Case 11).

When, however, not only the clearance, but also the concentration test show consistently normal values, there appears to remain little danger that recovery will not continue to completion. For this reason the concentration test is a desirable adjunct to the clearance in following the course of acute nephritis, particularly when the clearance approaches normal values.

On the other hand, as shown by Cases 4 and 5, the concentration test is much less sensitive than the clearance in indicating the beginning of recovery and its progress to approximate subjective and clinical completion.
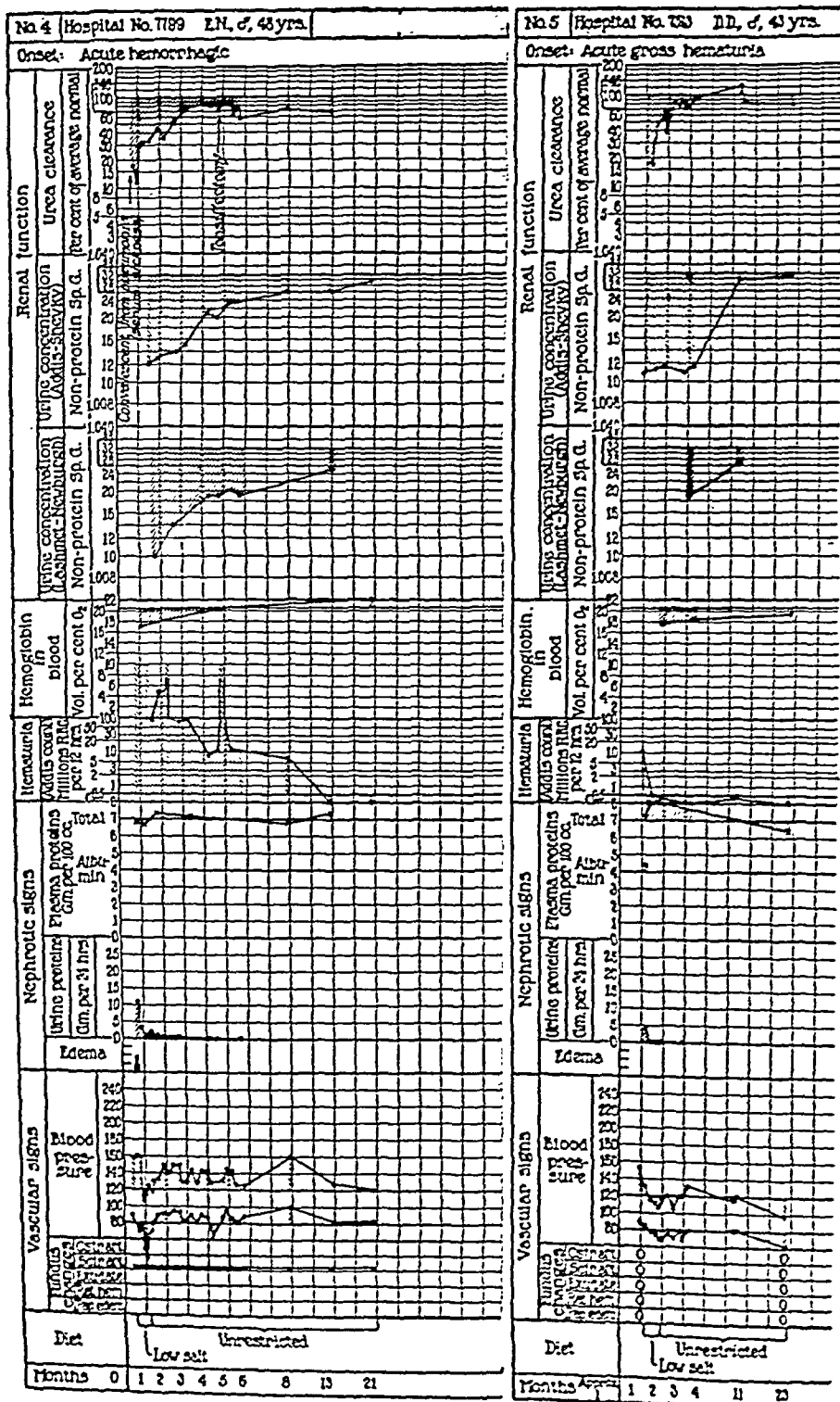
FIG. 7. COURSE OF TWO CASES RECOVERING FROM ACUTE NEPHRITIS

In each the urea clearance (top curve) rises to normal two or more months before the specific gravity in either concentration test (next 2 curves below) attains normal height. The first figure immediately after "Months" at the bottom indicates the time between apparent onset of nephritis and admission to hospital.
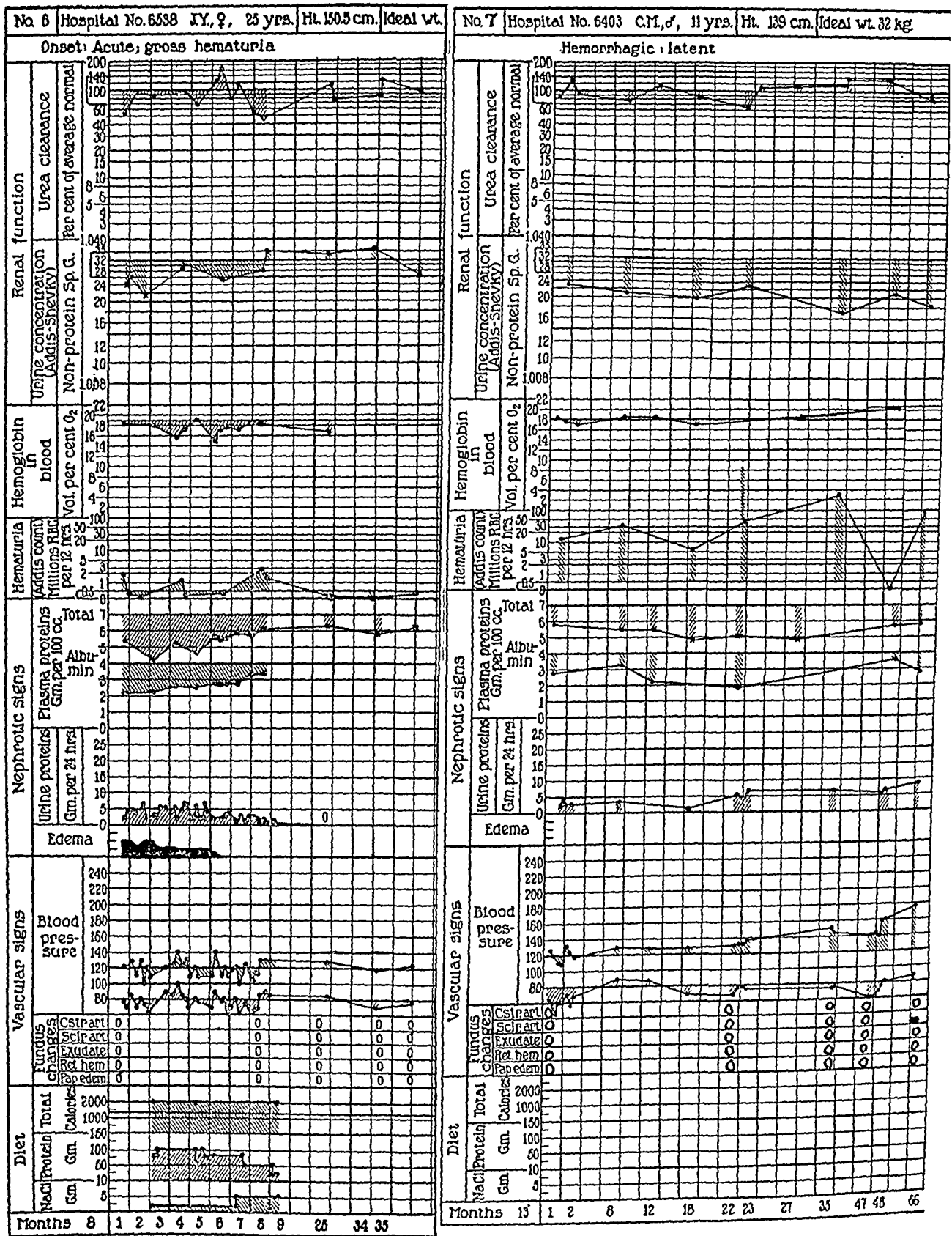
FIG. 8. UREA CLEARANCE AND ADDIS-SHEVKY SPECIFIC GRAVITIES DURING LATENT STAGE OF HEMORRHAGIC NEPHRITIS

Case 6, who eventually recovered, showed parallelism between urea clearance and Addis-Shevky concentration test. Case 7 has had normal urea clearance and low Addis-Shevky concentration test for 5 years. Continued hematuria, proteinuria, and subnormal plasma protein content confirmed the concentration test in indicating that some renal lesion persists.

Latent stage of hemorrhagic nephritis

In this stage, where recovery from initial nephritis has paused with the attainment of subjective well being, but with persistence of some signs, such as proteinuria, slight hematuria, or hypertension, the urea clearance usually returns to normal. The concentration test may also become normal, or it may continue to give low results. In Case 6, Figure 8, a patient who eventually recovered, parallelism between the two tests is seen, while in Case 7 a low concentration test has persisted for nearly five years, despite a consistently normal clearance. In this patient the persistence of hematuria and proteinuria indicates that the kidneys have not returned to normal.

The chronic active stage of hemorrhagic nephritis

It is in this stage (defined by Van Slyke, Stillman, *et al.* (1930) as that in which the disease has assumed a chronic character, but with the urea clearance still above 20 per cent of normal) that one is most likely to find parallelism between the urea clearance and the concentration test. A large proportion of the data presented in Figures 1 to 5 are from cases in this stage. The average specific gravities corresponding to given clearances are indicated by the locus of the broken line bisecting the fan-shaped area, included between the two lines bounding the field of the main body of results, in each of these figures. The wide range of variation from the average is indicated by the range of clearances lying within the field at the level of any given clearance. The degree of correlation in this stage is also illustrated by Cases 8 and 9, during the periods when their urea clearances were definitely above 20 per cent of normal (Figs. 9 and 10).

Advanced cases with fixation of urine specific gravity, while the urea clearance continues to show important variations in function

The fall from 20 or 30 per cent to 5 per cent of mean normal urea clearance has been found to correspond to a change from a clinical condition still compatible with occupational activity to one which ushers in uremia (Van Slyke, Stillman, *et al.* (1930)). The concentration tests, however, appear in this range to be relatively insensitive to variation. Thus in Figure 1 the cases with urea clearance between 5 and 20 per cent of mean normal show by the Lashmet-Newburgh test, specific gravities between 1.0075 and 1.012 scattered indiscriminately without apparent relationship to the urea clearance. A similar behavior of results of the other concentration tests is shown by Figures 2, 3–A, 4–A, and 5–A. The course of Case 9, Figure 10, beginning with the 17th month, of Case 10, Figure 11, throughout the period charted, and of Case 11, Figure 11, after the 6th month, illustrate the behavior of the two tests during the progress of advanced nephritis. The gradual fall of the urea clearances contrasts
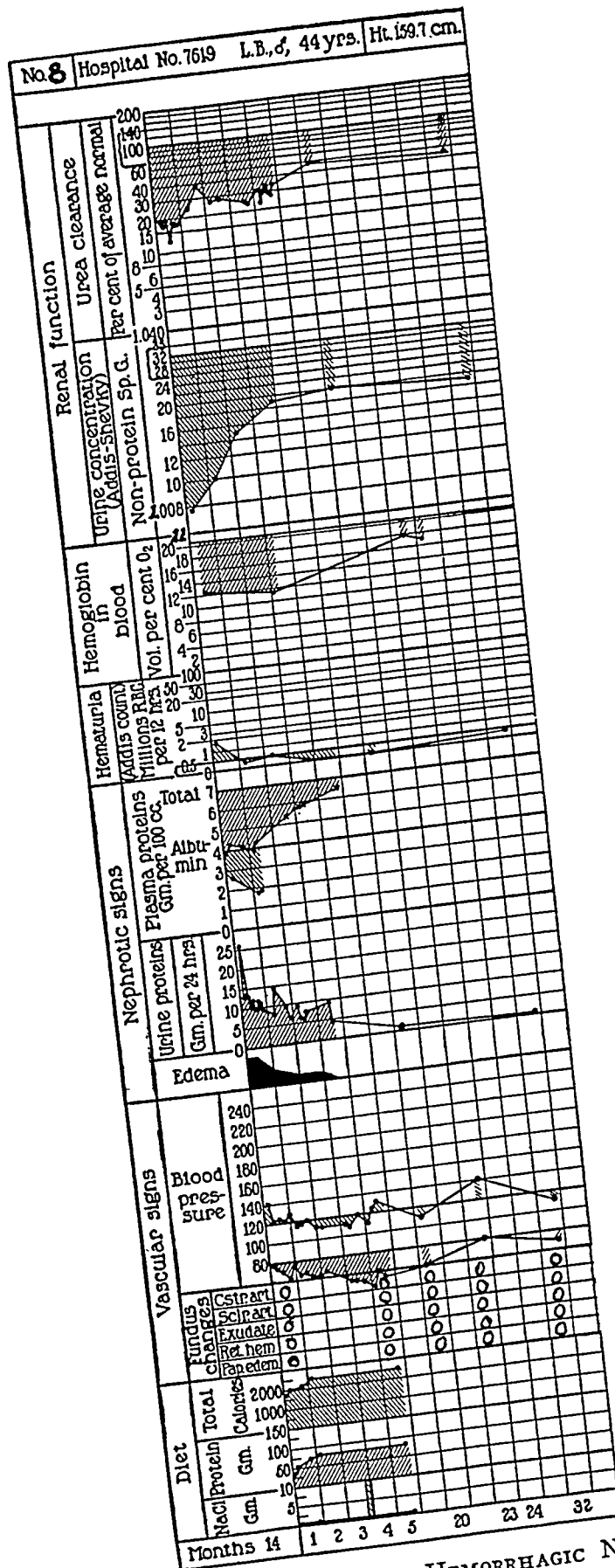
FIG. 9. CASE OF CHRONIC ACTIVE HEMORRHAGIC NEPHRITIS, SHOWING DEGREE OF CORRELATION BETWEEN CLEARANCE AND CONCENTRATION TESTS DURING PROGRESS OF THE DISEASE.
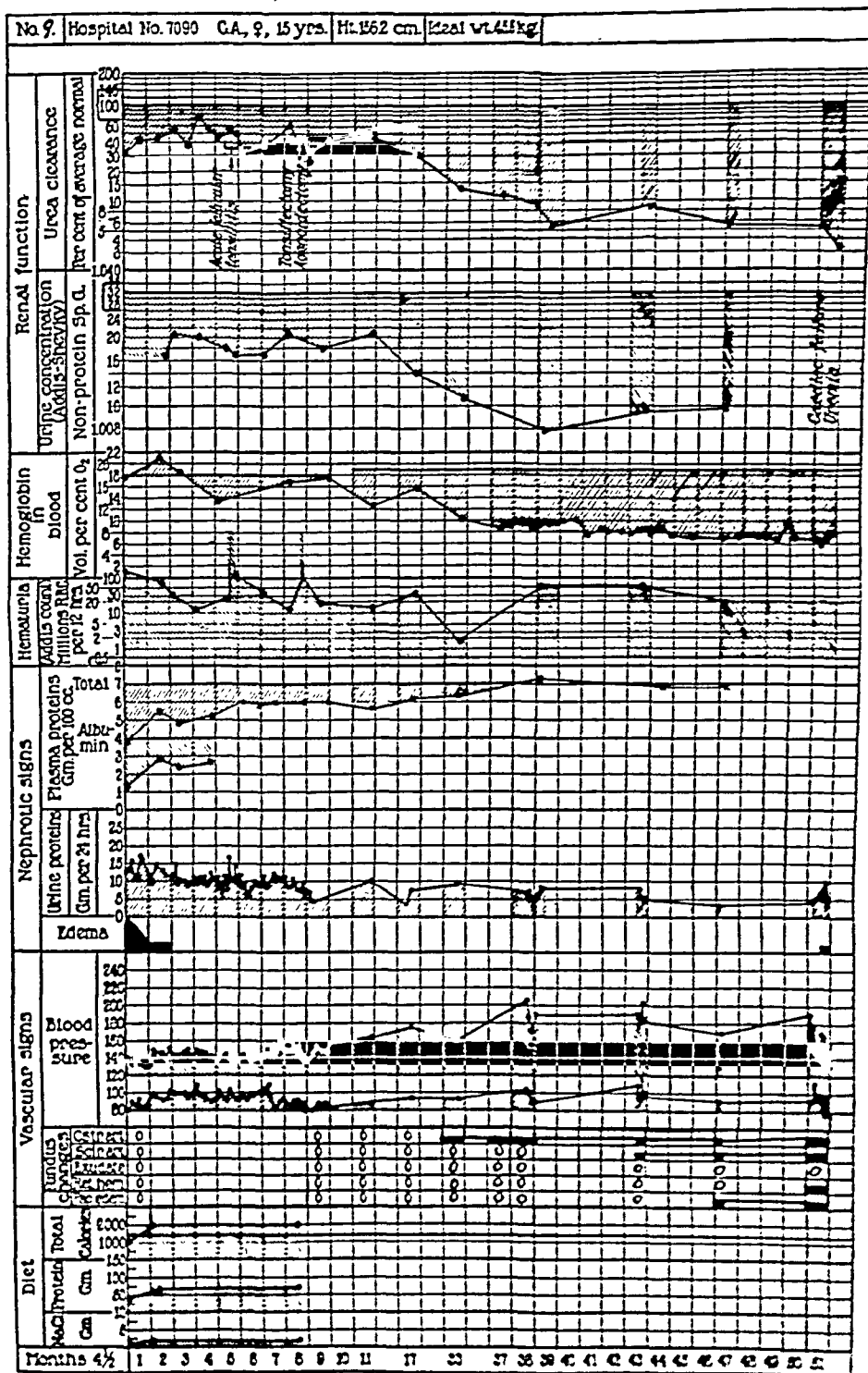
FIG. 10. CASE OF CHRONIC ACTIVE HEMORRHAGIC NEPHRITIS, SHOWING DE-
GREE OF CORRELATION BETWEEN CLEARANCE AND CONCENTRATION TESTS DURING
PROGRESS OF THE DISEASE.
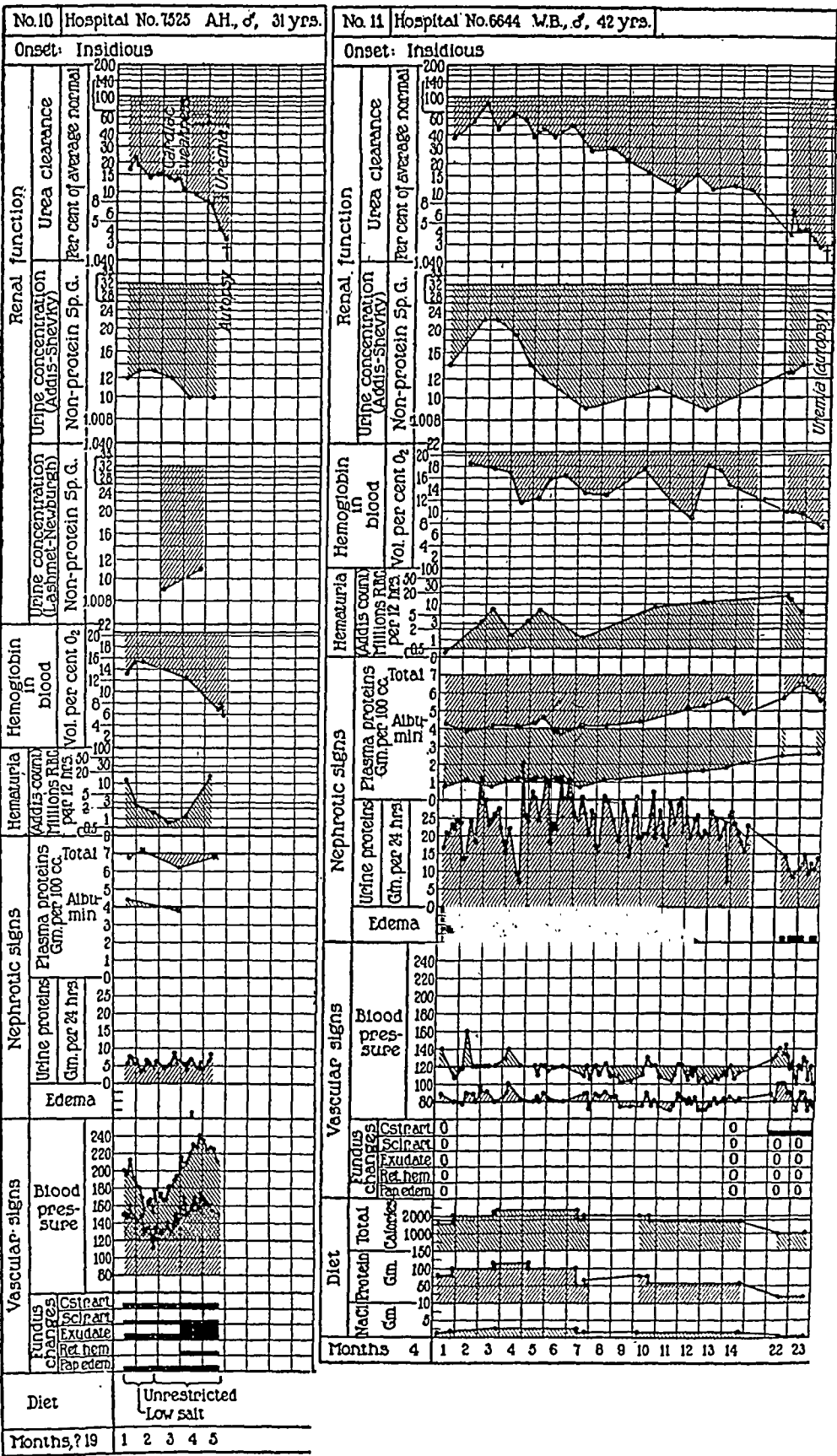
FIG. 11. Urea Clearance and Addis-Shevky Specific Gravity During
Progress of Advanced Nephritis in Two Cases

with the stationary condition of urine specific gravities which, having already reached the zone of minimum values, show no further significant changes.

While the urea clearance therefore continues to be of value in showing the progress of the disease and the margin of remaining renal function in advanced chronic nephritis, the concentration test does not.

Essential hypertension and nephrosclerosis

Cases of arteriosclerosis with normal function by the urea clearance are likely to show also normal function by concentration tests (Case 12, Figure 12).

As renal function fails, the clearance and concentration tests show the same relative behavior as in chronic hemorrhagic nephritis. There is some degree of parallelism until the clearance has fallen to 20 to 30 per cent of normal. By that time, however, the gravity has approached its minimal value, and can not be of much assistance in following the further course of the disease (Case 14, Figure 12).

Nephrosis

In nephrosis with slight or moderate diminution of urea clearance the concentration test may parallel the clearance (Cases 15 and 16, Figure 13). Whether nephrotic cases, like hemorrhagic cases, show exceptions to this parallelism we can not state, as the number of nephrotic cases observed has been too small.

Conditions invalidating the concentration test

That various physiological and pathological conditions of extrarenal origin can at times invalidate the results of concentration tests has been well recognized by the authors of these tests. Addis and Shevky (1922) pointed out that extrarenal influences of endocrine, nervous, or metabolic origin could increase the water output, or decrease the solid output, during the period of their test, and produce lowering of specific gravity simulating that caused by renal deficit. Among the metabolic factors they mentioned unusually low nitrogen or salt content of the diet. Usually, however, according to the present authors' experience, the normal subject automatically adjusts his water intake so that these factors appear only rarely to invalidate the results.

Specific disturbing factors pointed out by Volhard (1918) are unusual desiccation or water flooding of the subject during the days preceding the test, storage or excretion of edema fluid in subjects of the nephrotic type during the test, and cardiac decompensation.

Mosenthal (1930) states: " A low fixed specific gravity . . . is found in many widely varying conditions; marked anemia, elimination of edema,
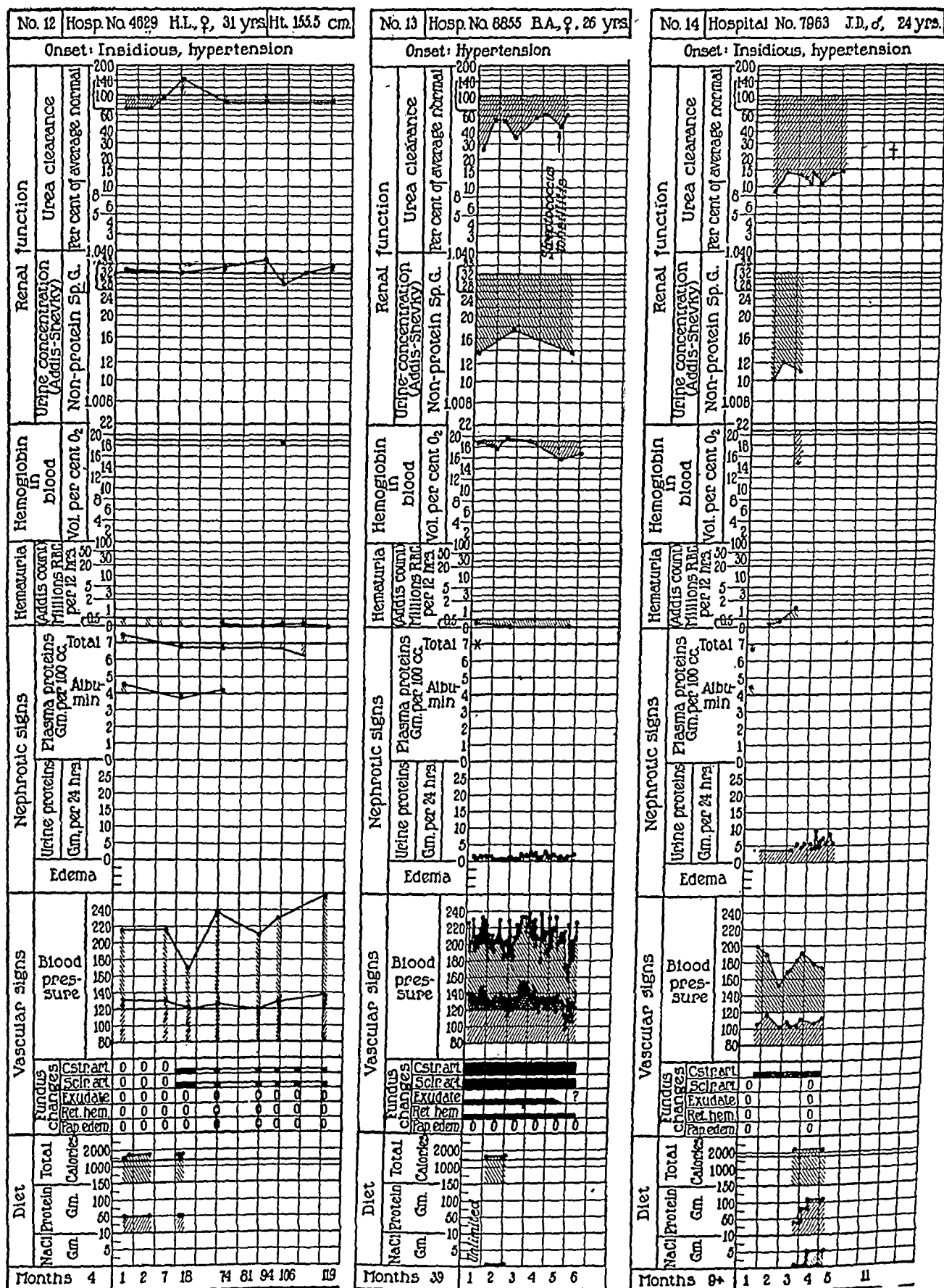
FIG. 12. UREA CLEARANCE AND ADDIS-SHEVKY SPECIFIC GRAVITY IN THREE CASES BEGINNING AS ESSENTIAL HYPERTENSION

In Cases 13 and 14 proteinuria has developed, and the function tests indicate renal destruction moderate in Case 13, advanced in Case 14.
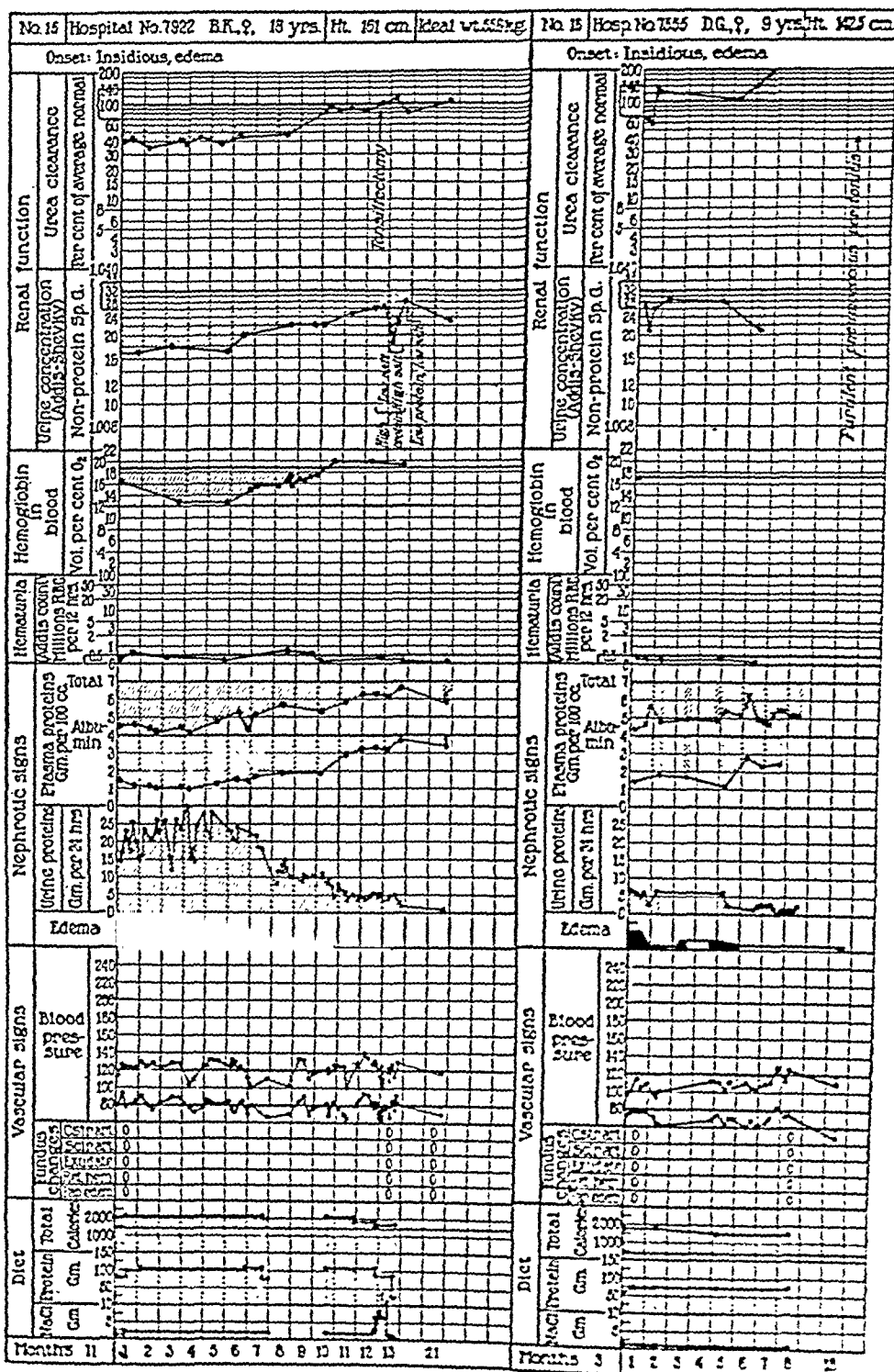
FIG. 13. TWO CASES OF NEPHROSIS SHOWING THE DEGREE OF CORRELATION BE-
TWEEN THE CLEARANCE AND CONCENTRATION TEST

The urine specific gravities of Case 15 during the 13th month show the unexpected
influence of increased salt intake in lowering the specific gravity obtained in the con-
centration test (see text, p. 990).

pyelitis, polycystic kidney, prostatic hypertrophy, urethral stricture, paralysis of the bladder (as in tabes dorsalis or tumor of the cord), and others. A lower fixed specific gravity occurs in diabetes insipidus."

Observations made on Case 15 (Figure 13) during the 13th month after admission illustrate the unpredictable influence which extrarenal factors may have on the results of a concentration test. This patient, nearing recovery from degenerative Bright's disease, could concentrate to 1.027 when on her usual diet, which was fairly high in protein, but contained only 1 to 2 grams of salt per day. Addis and Shevky (1922) reported that in normal subjects low protein or low salt intake sometimes caused subnormal specific gravities in the concentration test. We consequently expected that raising the salt intake might increase the specific gravity from a minimal normal to the usual full normal value (1.030 to 1.034). On the contrary, when the salt intake was raised to 10 to 12 grams on two occasions the specific gravity fell to 1.018 and 1.023 respectively. Apparently the diuretic effect of the salt caused the excretion of relatively more water than solids.

Despite the possible interference of such invalidating factors, however, it is our experience that, when signs of renal lesion exist or have existed, consistently low results in concentration tests afford presumptive evidence that some of the renal damage persists, even though the urea clearance is normal.

The relative influence of different urinary constituents on the specific gravities obtained in the concentration tests

Figure 14 indicates a close correlation between the mineral salt content and the specific gravity of urine voided under the conditions of the Lashmet-Newburgh concentration test. That the mineral salts are chiefly responsible for the extent by which the urine gravity exceeds that of water appears from the following considerations.

From Figure 14 it is evident that with increase in specific gravity the total base concentration, in milli-equivalents, usually increases at about the same rate as the millimolar urea concentration of the urea, and that a urine of specific gravity 1.030, obtained under the conditions of the test, has an average mineral content of about 400 milli-equivalents per liter and a urea content of about 400 millimols. The specific gravities in Table I (corrected to apply to water at the same temperature as unity) are taken from Landolt-Börnstein's " Tabellen."

It is obvious from Table I that 1 milli-equivalent of base combined with the anions present in urine raises the specific gravity from 2 to 5 times as much as 1 millimol of urea. One gram equivalent of base combined with Cl or acetate anion has about 2 times the effect of one gram molecule of urea, base with lactate, SO_4 or HPO_4 has about 3 times, and

TABLE I

Specific gravities at 20° C. corrected to water at same temperature (Landolt-Börnstein)

Solute	Concentration	Specific gravity
Urea......................	0.4 molar	1.007
NaCl.....................	0.4 milli-equivalent	1.016
Na₂SO₄...................	0.4 milli-equivalent	1.025
KCl......................	0.4 milli-equivalent	1.018
K₂SO₄....................	0.4 milli-equivalent	1.027
MgCl₂....................	0.4 milli-equivalent	1.015
MgSO₄...................	0.4 milli-equivalent	1.024
Na₂HPO₄.................	0.4 milli-equivalent	1.026
KH₂PO₄..................	0.4 milli-equivalent	1.038
Na acetate...............	0.4 milli-equivalent	1.015
Na lactate...............	0.4 milli-equivalent	1.021

base with H_2PO_4 about 5 times the effect of one mol of urea. Figure 14 indicates that in urine excreted under the conditions of the Lashmet-Newburgh test, about ⅓ of the base is present as chlorides, which in 0.4 N concentration raise the specific gravity by 0.016 or 0.017 units. The other ⅔ must be in great part in the group of salts which in 0.4 N concentration cause specific gravity increases of 0.025 or more units. If we assume that, in the urine of specific gravity 1.030, the total base is 0.4 normal, and ⅓ of the salts are present as chlorides, ⅔ as sulfates and phosphates, the specific gravity due to the minerals would be about 1.023. Adding 0.007 due to the urea gives the entire gravity value 1.030. The calculation is, of course, only a rough approximation, but it serves to show that in urines collected under the conditions of this test, about ¾ of the rise in specific gravity above that of water may ordinarily be attributed to the mineral salts, and about ¼ to urea, with other organic solids of relatively slight influence.

In cases with normal urea clearance and low urine gravities, the latter are attributable to the fact that the patients excrete urine containing a low concentration of nonprotein solids, of which the most important with regard to effect on specific gravity are the mineral salts. In the Lashmet-Newburgh tests the low gravities appear not due to high volumes, for the urine volumes in these cases have not been high (hollow circles to left of specific gravity 1.026 line in Figure 15). The low gravities must therefore be attributed to a low total excretion of salts. in the 2-hour period. In the cases with normal clearance and low Addis-Shevky concentration tests, however (Figure 16), the 12-hour night urines, although sometimes normal in volume, were often of large volume. The low gravities in some of these cases therefore appear attributable to nocturnal polyuria, in others to a low output of salts.
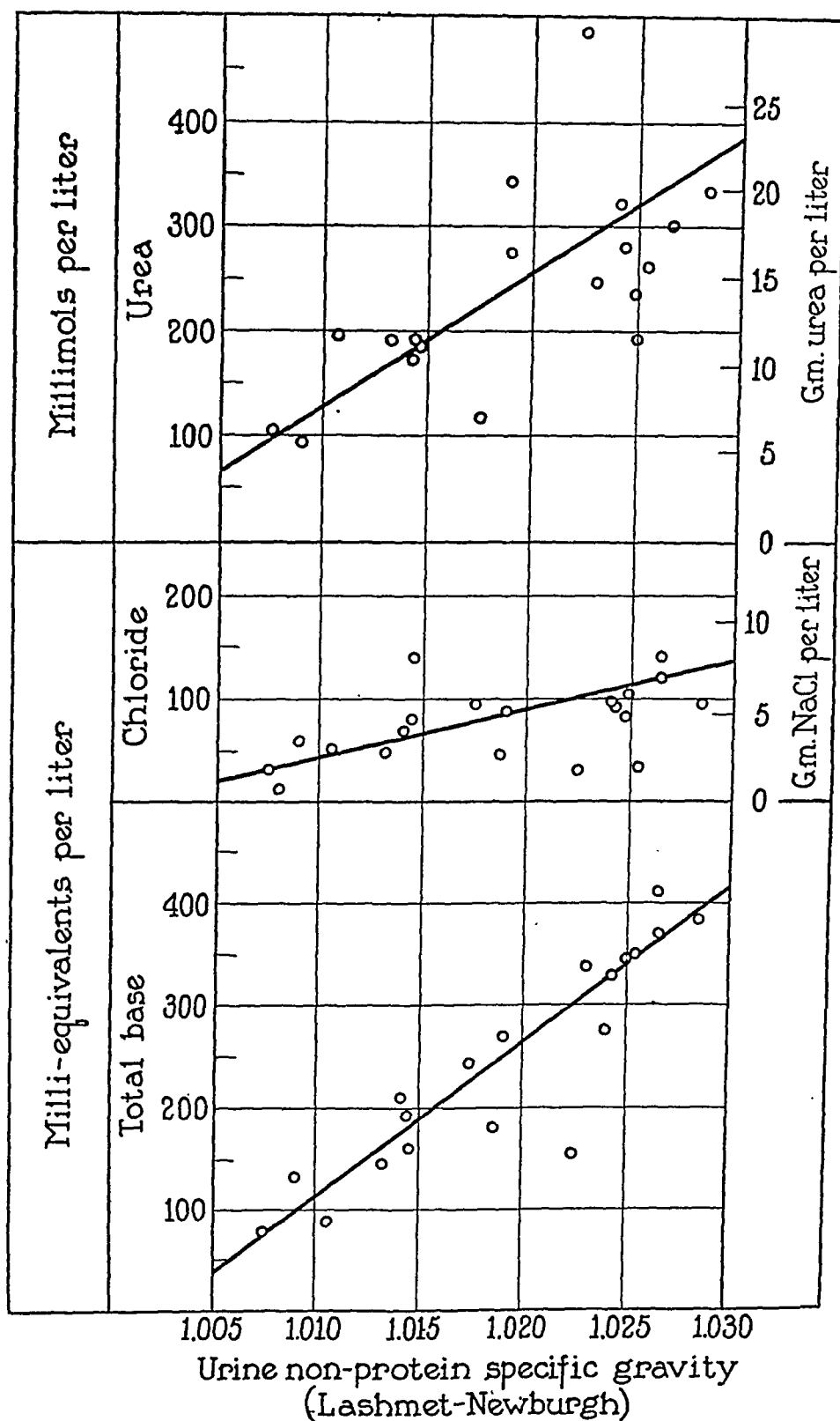
FIG. 14.   RELATIONSHIP OF URINE SPECIFIC GRAVITY TO UREA, CHLORIDE, AND
TOTAL BASE CONCENTRATIONS

Lashmet-Newburgh
concentration test

Addis-Shevky
concentration test
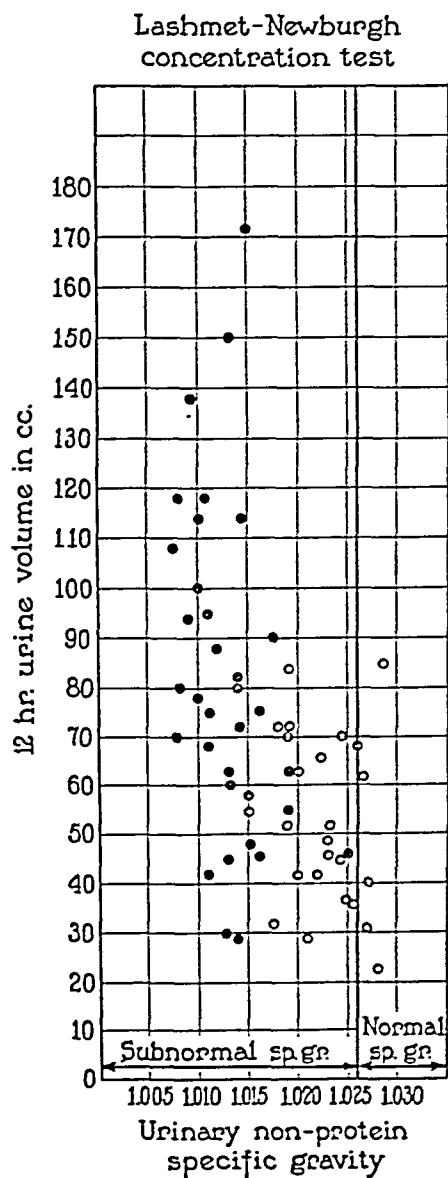
•=Blood urea clearance below 75% of mean normal.

o=   •   •   •   75% of mean normal or above.
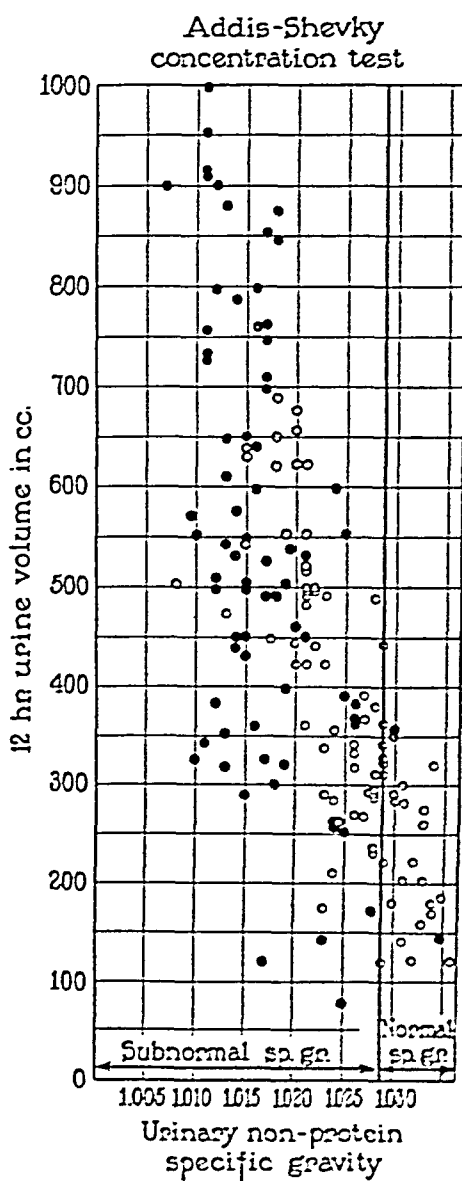
FIG. 15   FIG. 16

FIG. 15. URINE VOLUMES IN LASHMET-NEWBURGH TEST COMPARED WITH SPECIFIC GRAVITIES; IN CASES INDICATED BY •, WITH UREA CLEARANCES BELOW THE NORMAL RANGE, AND IN CASES, INDICATED BY O, WITH NORMAL OF HYPERNORMAL UREA CLEARANCES.

FIG. 16. URINE VOLUMES IN ADDIS TEST COMPARED WITH SPECIFIC GRAVITIES; IN CASES INDICATED BY •, WITH UREA CLEARANCES BELOW THE NORMAL RANGE, AND IN CASES, INDICATED BY O, WITHIN OR ABOVE THE USUAL NORMAL RANGES.

attached the metal scale divided into 50 divisions between the knife-edge fulcrum and the point of suspension of the beam. The solid glass plummet is of 10 cc. volume, so that a change of 0.001 in specific gravity of the liquid tested changes the load by 10 mgm. A 500 mgm. rider is used on the beam, so that each division represents a specific gravity change of 0.001. The balance is sensitive to a shift of half a division by the rider. The balance is adjusted to indicate 1.000 specific gravity when the fluid is water at the chosen standard temperature, 20° in the case of our work.[6]

FIG. 17. WESTPHAL SPECIFIC GRAVITY BALANCE MODIFIED FOR ROUTINE USE WITH URINE

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[6] The balance was made for us from an ordinary Westphal balance by Eimer and Amend, of New York.

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FIG. 17. WESTPHAL SPECIFIC GRAVITY BALANCE MODIFIED FOR ROUTINE USE WITH URINE

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[6] The balance was made for us from an ordinary Westphal balance by Eimer and Amend, of New York.

STUDY OF THE BLOOD IN CHRONIC RESPIRATORY DIS-EASES, WITH SPECIAL REFERENCE TO THE VOLUME OF THE BLOOD[1]

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The character of the changes in the blood in cases of chronic pulmonary diseases has not been settled. Although patients with respiratory disturbances often have the appearance of those with polycythemia, it is questionable whether there is an actual increase in the blood and cell volume. Some investigators believe that the blood in these cases shows a polycythemic picture, which they believe to be due to interference with the proper pulmonary exchange of oxygen, resulting in a low tension of oxygen in the arterial blood which in turn overstimulates the bone marrow. The usual response of the blood to low oxygen tensions obtained for short periods under experimental conditions has been studied extensively. But investigations of the changes occurring in cases of chronic pulmonary diseases with or without anoxemia due to faulty pulmonary exchange are not available except in a few isolated cases. We have attempted to clarify this problem by studying a group of cases with chronic pulmonary diseases; determining not only the characteristics of the erythrocytes and the blood volume but also correlating these findings with various factors such as arterial oxygen saturation, the pulmonary capacity and its sub-divisions and the duration and severity of the disease. We feel that these observations of the volume and morphology of the blood are enhanced by other studies, to be reported later, which we have made on these patients in order better to classify and understand their functional pathology.

Well established standards of the normal morphology of the red blood cells are available in the literature, but the values for the blood volume reported by different observers, using similar technique and experimental conditions, vary markedly. We have collected in Table I the values for

TABLE I

Values for the plasma and blood volumes collected from the literature

(Normal males)

	Number of cases	Dye	Mixing time	Hemoglobin	Cell volume	Plasma		Blood	
						Total	Per kgm.	Total	Per kgm.
			minutes		*per cent*	*liters*	*cc.*	*liters*	*cc.*
Mendershausen (1)*......	16	Congo red	4		44.1	2.25	39.0	4.13	71.7
Seyderhelm & Lampe (2)..	6	Trypan red	3–6	86%	47.9	2.58	44.1	4.93	84.7
Rusznyák (3).......	8	Trypan red	3–6	92%		2.84	44.7	5.32	83.3
Rowntree & Brown (4)......	49	Congo red	3–6	16.4 grams	42	3.48	51	6.04	88.6
Uhlenbruck (5).......	7	Congo red	3–6			2.77	41.4	5.34	79.6
Sparks & Haden (6).......	10	Congo red	4			2.63	34.4	4.96	64.9

* Bibliography number.

the plasma and blood volume in normal male subjects reported in the literature, including only those reports dealing with the larger number of cases. All these determinations, in normal male subjects of various age groups were made by injecting a dye into the peripheral blood stream according to the method first introduced by Keith, Rowntree and Geraghty (7). The plasma volume has been variously estimated from 34 cc. to 51 cc., per kilogram and the volume of blood, from 72 cc. to 89 cc. per kilogram of body weight. Fleischer-Hanson (8), who objects to the above procedure because of inadequate mixing of the blood and dye in the time allowed, has the subject perform the so-called "mixing movements" and finds the volume of the blood as low as 49 cc. per kilogram. Because of this wide variation it was felt necessary to determine our own standards for normal individuals in order to have a basis for evaluating our study of pathological cases. A review of the literature also indicates that one must allow generous limits for normal variation.

METHODS

In this investigation the dye method of Keith, Rowntree and Geraghty (7) with the modifications as described by Hooper, Smith, Belt and Whipple (9) was used for determining the plasma volume. All the observations were made with the patient in the recumbent position, following a fast of twelve hours. A needle was inserted into the antecubital vein and 12 to 15 cc. of blood were removed without stasis. About 3 cc. of the blood were placed in an oxalated tube for hemoglobin determination, while the remaining 8 to 10 cc. of blood were placed in a centrifuge tube accurately calibrated to tenths of a cubic centimeter and containing 2 cc. of 1.6 per cent solution of sodium oxalate. The tube was stoppered and inverted several times to prevent clotting. Without removing the needle from the vein, a 1.5 per cent solution of brilliant vital red, freshly prepared and filtered, was injected into the blood stream. In each case four milligrams of the dye per kilogram body weight were introduced. Four minutes after the injection, 8 to 10 cc. of blood were removed from the antecubital vein of the opposite arm. This blood was placed in another graduated centrifuge tube containing 2 cc. of 1.6 per cent solution of sodium oxalate. This was stoppered, inverted and, along with the other tube, was centrifuged at 2700 revolutions per minute for 45 minutes. Then the hematocrit and other readings necessary for the calculation of the plasma volume were made. The standard, made from the filtered solution, and the unknown solution were then prepared for colorimetric comparison. The volumes of the plasma and blood were calculated according to the formulas derived by Hooper, Smith, Belt and Whipple (9). In all cases the hemoglobin concentration was determined on the first sample of blood by measuring the oxygen combining power according to

Van Slyke's method (10). It is possible to calculate the corpuscular volume and hemoglobin per 100 cc. of blood. We have not encountered a single reaction to the injection of the dye in 55 determinations.

The oxygen content and combining power of the arterial blood were determined in the Van Slyke manometric apparatus.

The total pulmonary capacity and its sub-divisions were measured according to the methods used in this clinic (11, 12).

DETERMINATIONS ON NORMAL SUBJECTS

The volumes of the plasma and blood were estimated on 25 normal male subjects, who were students in this University. No selective criteria as to physical characteristics were used save that the subjects were free from any discernible disease. A clinical history was obtained from each individual and a physical examination made.

The physical characteristics and the values for the blood volume and its components of these subjects are summarized in Table II. The weight observed in this group corresponds closely with the average ideal weight calculated from age and height.

TABLE II

The volumes of the plasma and blood in 25 normal males with age and physical characteristics of the subjects

(Dye method—Brilliant vital red)

	Mean	Standard deviation	Coefficient of variation	Range
Age, *years*................	26 ± .31‡	2.26± .22‡	8.7	22 − 32
Weight, *kgm*..............	76.2 ± 1.22	9.07± .87	11.9	60.6 − 104
Height, *cm*................	178.2 ± .55	4.07± .39	2.3	170 − 186.5
Surface area, *square meters*...	1.93± .02	.11± .01	5.7	1.73− 2.19
Plasma volume, *liters*........	3.22± .06	.42± .04	13.0	2.21− 4.06
Plasma/kgm., *cc*............	42.8 ± .59	4.4 ± .42	10.3	34.7 − 50.3
Plasma/square meter, *liters*...	1.69± .02	.17± .02	10.1	1.28− 1.88
Blood volume, *liters*.........	5.93± .10	.77± .08	12.9	3.95− 7.49
Blood/kgm., *cc*.............	79.1 ± .97	7.19± .69	9.1	62.1 − 91.1
Blood/square meter, *liters*....	3.10± .04	.30± .03	9.8	2.28− 3.42
Cell volume, *liters*..........	2.69± .05	.39± .04	14.5	1.66− 3.38
Cells/kgm., *cc*.............	35.8 ± .49	3.62± .06	10.1	26.0 − 44.7
Cells/square meter, *liters*.....	1.40± .02	.16± .02	11.4	.96− 1.68
Total hemoglobin, *grams*.....	982 ±20.2	149.9 ±14.3	15.2	590 −1216
Hemoglobin/kgm., *grams*.....	12.9 ± .20	1.47± .14	11.3	9.3 − 15.3
Hemoglobin/square meter, *grams*..................	499 ± 8.8	64.9 ± 6.2	13.0	341 − 590
Hemoglobin per 100 cc., *grams*	16.2 ± .11	.84± .08	5.1	14.9 − 17.7
Plasma, *per cent*............	54.3 ± .32	2.38± .23	4.3	47.9 − 58.4
R.B.C., *per cent*............	45.1 ± .31	2.29± .22	5.1	40.9 − 51.3

‡ Probable error.

The mean value of the *plasma volume* was 3.22 liters with variation between 2.21 and 4.06 liters, while the plasma per kilogram of body weight was 42.8 cc., a value just about midway between the lowest and the highest values reported in the literature.

The *blood volume* was observed to vary markedly both in total volume and volume per kilogram of body weight. The mean value of the former was 5.93 liters varying from 3.95 to 7.49 liters, while that of the latter was 79.1 cc. with variation between 62.1 and 91.1 cc. The mean value for blood volume per kilogram of body weight was again found to be about midway between the extremes in the literature.

The total volume of the cells and total hemoglobin varied markedly from individual to individual. The mean value for the *cell volume* was 2.69 liters while the value for each kilogram of body weight was 35.8 cc. The mean value for the *hemoglobin per kilogram of body weight* was 12.9 grams, a figure almost two grams lower than that reported by Rowntree and Brown (4) in a similar group of cases.

The mean value for the *red blood cell percentage* was 45.1 with variation between 40.9 and 51.3. Determinations of *hemoglobin per 100 cc. of blood* gave a mean value of 16.2 grams. These results correspond very closely with those collected from papers in the literature and reported by Wintrobe (13), who found in 381 cases (all males) that the average value for the hematocrit was 46.3 per cent and in 583 cases the hemoglobin per 100 cc. of blood was 16.0 grams.

The correlation of the blood volume and its components to body weight and body surface area is given in Table III. The most significant correlation exists between the body weight and the volume of the blood and its

TABLE III

Correlation of blood volume with various body measurements

	Correlation coefficient
Total blood volume to body weight	.7297 ± .0631‡
Total blood volume to surface area	.6756 ± .0733
Total plasma volume to body weight	.6954 ± .0697
Total plasma volume to surface area	.6387 ± .0798
Total cell volume to body weight	.8014 ± .0483
Total cell volume to surface area	.7840 ± .0519
Total hemoglobin to body weight	.7700 ± .0549
Total hemoglobin to surface area	.7648 ± .0560

‡ Probable error.

components. This finding indicates that knowing the body weight of an individual in kilograms one can predict the total blood volume with a fair degree of accuracy by means of the regression formula derived from the coefficient of correlation.[2] From the predicted blood volume and the nor-

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[2] The regression formula is:

$$\text{Total volume} = (\text{Kilograms body weight} \times 0.06) + 1.41.$$

mal mean values for hematocrit and hemoglobin per 100 cc. of blood, one can calculate the expected cell and plasma volumes and the total hemoglobin. Rowntree and Brown (4) found the best correlation between surface area of the body and the plasma and blood volume.

The total plasma, blood and cell volume and the total hemoglobin of the 25 normal subjects were calculated from the regression formulas and compared with the observed values for the purpose of establishing the normal range of variability. Of the observed blood volumes 92 per cent fell within the limits plus and minus 15 per cent of the calculated values; while of the observed plasma and cell volumes and total circulating hemoglobin 88 per cent were within this same limit. Individual variations around plus and minus 15 per cent of the calculated may not be significant but when the mean value for a group of pathological cases approaches this limit, the difference becomes of some importance.

DETERMINATIONS ON CASES WITH CHRONIC PULMONARY DISEASES

Literature

Blood volume determinations have been made both by the carbon monoxide and dye methods on patients with chronic respiratory diseases. In five cases with chronic bronchitis, emphysema and asthma, studied by Plesch (14) the average blood volume by the carbon monoxide method was 72 cc. per kilogram of body weight. This was 35 per cent greater than the values which he found in nine normal subjects. These values are of course low when compared with those that one obtains by means of the dye method; a difference found repeatedly by many investigators. Uhlenbruck (5) using the dye method (congo red), confirmed the results of Plesch. He estimated the volume of the blood and plasma in 7 normal males and found mean values of 79.6 cc. and 41.4 cc. per kilogram of body weight respectively. In 8 male patients with emphysema and asthma this investigator found an average blood volume of 101 cc. per kilogram of body weight with extreme variations of 78 and 147 cc. and an average plasma volume of 53 cc. per kilogram, an increase in blood volume of 27 per cent and an increase in the plasma volume of 28 per cent. Lemon (15) reported 12 cases (11 males and 1 female) with pulmonary emphysema, in which he determined not only the volume of the plasma and blood but also the arterial oxygen saturation, the vital capacity and the characteristics of the erythrocytes. He found that the mean value for the blood volume per kilogram of body weight was only slightly higher (4 cc.) than that of the normal group. In several cases there was evidence of increase in the concentration of the blood, i.e. increase in the volume of cells without increase in the blood volume. Along with the concentration of the blood he observed a definite increase in the hemoglobin content of the erythrocytes. Arterial blood was obtained in 7 cases and, in all, the satu-

ration was less than 95 per cent. No correlation was demonstrated between the decrease in the oxygen saturation of the arterial blood and the alterations in the blood volume or between decrease in vital capacity and changes in the blood volume. None of the cases which he studied simulated polycythemia vera. So far as we can find no one has reported determinations of blood volume in a group of cases with pulmonary fibrosis.

Material

Determinations of plasma and blood volume and morphology of the cells were made in 16 cases with chronic pulmonary emphysema and in 14 cases of pulmonary fibrosis. The major purpose of this study was to attempt to correlate these findings with the clinical condition of the patient, the oxygen saturation of the arterial blood and the pulmonary capacity.

Emphysema. The 16 patients in this group had varying degrees of emphysema associated with chronic bronchitis, bronchiectasis or asthma. The physical characteristics, symptoms and signs, with interpretations of the roentgenograms of the chest are given in Table IV. The age of the individuals varied from 31 to 59 years. None of the patients was particularly obese; many of the subjects weighed less than their " ideal " weight calculated according to the height and age. The average weight of the group was 6 kilograms below that of their " ideal " weight. All of the patients except one complained of cough. Dyspnea was a symptom of all the subjects, and in two it was present during rest. All but three had asthmatic attacks. The duration of the symptoms varied from 1 to 25 years. None of the patients complained of nervous or psychic symptoms. Cyanosis was observed in half the cases; clubbing of the fingers was found in 6 of the 16 subjects. The spleen was not palpable in any of these individuals. The degree of emphysema was estimated from the relative values of the residual air and the total pulmonary capacity. The blood pressure was within normal limits in all subjects. The electrocardiogram was normal in all except Case 1, in which there was evidence of myocardial damage, and in Cases 13, 14 and 15, in which left ventricular preponderance was indicated.

Fibrosis. In 10 of the 14 cases, the fibrosis was suspected to be due to inhalation of silica. The etiology of the fibrosis in the remaining cases is not known. One patient had arteriosclerotic heart disease which was well compensated at the time of the examination; another, Case 22, had a secondary anemia. The clinical observations in this group of patients are summarized in Table V. The age of the subjects varied from 32 to 65 years. The average " ideal " weight of the group was 68.6 kilograms as compared with that of the observed which was 67.7 kilograms. A high percentage of the patients complained of cough and dyspnea, while only two of them (Cases 19 and 24) gave a history of asthma. The duration

TABLE IV

Clinical observations made on 16 cases of pulmonary emphysema

Case	Age	Wt.	Height	Cough	Dyspnea	Asthma	Cyanosis	Duration of symptoms	Clubbing of fingers	Degree of emphysema*	Diagnosis	X-ray findings
	years	kgm.	cm.									
1	51	52.0	157	++	++	0	0	1 year	++	+++	Bronchitis Emphysema	Increased linear markings in both lung fields.
2	54	60.8	174.5	+	++	++	+	Asthma 20 years	0	+++	Asthma Emphysema	Slight increase in linear markings of both lung fields.
3	41	58.4	179.5	++	+++	++	+	2 years	0	+++	Bronchiectasis Asthma Emphysema	Increased linear markings. After lipiodal definite evidences of bronchiectasis.
4	44	54.0	157.5	++	++	+++	+	2 years	−	++	Asthma Emphysema	Increased lung markings; local areas of increased radiability; slight amount of rib flaring.
5	40	49.7	160.5	+++	++	++	0	2 years	+	+	Bronchitis Asthma Emphysema	Pulmonary markings were greatly exaggerated.
6	38	81.2	159.5	++	++	++	0	?2 years	++	++	Bronchitis Asthma Emphysema	Some increase in linear markings.
7	41	54.4	152.5	+	+	++	0	Every winter 10 years	+	++	Bronchitis Asthma Emphysema	Linear markings increased. Increased radiability of both lung fields.
8	31	46.0	159.0	++	++	++	+	3 years	+	++	Emphysema Asthma	Exaggeration of lung markings, particularly in the right lung.

* Established according to alterations in the pulmonary capacity and its sub-divisions.

TABLE IV (continued)

Case	Age	Wt.	Height	Cough	Dyspnea	Asthma	Cyanosis	Duration of symptoms	Clubbing of fingers	Degree of emphysema*	Diagnosis	X-ray findings
	years	kgm.	cm.									
9	32	42.6	150.0	0	+	++	0	12 years	0	++	Asthma (Allergic) Emphysema	Increased radiability at the lung bases; flaring of rib cage.
10	44	79.4	177.2	++	++	++	+	13 years	-	+++	Emphysema Asthma	General increase in linear markings. Diaphragms low; slight amount of rib bulging.
11	59	83.2	169.5	+	+++	++	++	25 years	0	+++	Asthma Emphysema	Large lung fields; increased lung markings; increased radiability at the lung bases.
12	52	67.4	176.0	+++	++	++	0	5 years	0	++	Bronchitis Asthma Emphysema	Increased radiability of lung fields; moderate amount of rib flaring; low diaphragms.
13	49	76.6	171.5	++	++	++	+	2 years	0	++	Bronchitis Asthma Emphysema	Increased linear markings. Hilus shadows enlarged.
14	36	78.0	165.0	++	+	+	+	14 years	+	+	Bronchiectasis Emphysema Asthma	Increased linear markings; increased radiability of lung bases. Definite saccular bronchiectasis at both bases after lipiodol.
15	48	69.1	163.4	+	+++	0	0	3 years	0	+	Bronchitis Emphysema	Slight increase in linear markings; Slight flaring of rib cage.
16	45	57.0	172.5	++	+	0	0	Several years	0	+	Bronchitis Emphysema	Exaggerated lung markings, more pronounced on the right associated with pleural thickening.

TABLE V

Clinical observations made on 14 cases of pulmonary fibrosis

Case	Age (years)	Wt. (kgm.)	Height (cm.)	Cough	Dyspnea	Dust exposure	Cyanosis	Clubbing of fingers	Duration of symptom	Examination of chest	Diagnosis	X-ray findings
17	45	70.0	166.0	+	++	Coal miner 5 years	0	0	2 years	Muscial râles at bases	Fibrosis of lungs	Increased linear markings.
18	41	70.8	177.0	++	0	0	0	0	?	0	Fibrosis (no etiological factor)	Pronounced exaggeration of lung markings and feathering.
19	48	48.8	154.0	++	+++	Sand blasting 6 years	+	+	5 years	Coarse bubbling râles	Fibrosis Bronchitis	Marked increase in linear markings. Hilus shadows enlarged.
20	47	66.8	165.4	0	+	Sand blasting 2 years	0	0	2 years	Negative	Fibrosis Emphysema (?)	Both lung fields show diffuse mottling and feathering. Hilus shadows increased. Bases show increased radiability.
21	47	56.2	152.6	+	++	Coal miner 26 years Some drilling or rock	0	0	3 years	Percussion note impaired with fine râles over right lung posteriorly	Fibrosis	Upper lung fields show massive nodular shadows. Increased radiability of bases.
22	42	73.2	174.0	+++	++	Salesman Spraying of paints & lacquer	0	0	3 years	Fine râles at both bases	Fibrosis Secondary anemia	Increased linear markings. Slight mottling in both lungs.
23	36	55.0	163.0	+	++	Sand blasting 5 years	+	0	3 years	Scattered transient râles	Fibrosis	Mottling throughout both lungs.

TABLE V (continued)

Case	Age years	Wt. kgm.	Height cm.	Cough	Dyspnea	Dust exposure	Cyanosis	Clubbing of fingers	Duration of symptoms	Examination of chest	Diagnosis	X-ray findings
24	43	55.0	158.0	+++	+++	Silica for 13 years	0	0	2 years	Bronchial breath sounds at left apex and right base. Râles both bases	Fibrosis	Coarse mottling throughout both lungs.
25	33	76.0	178.5	+	++	Sand blasting 5½ years	+	0	1 year	Few scattered râles	Fibrosis	Diffuse coarse mottling throughout both lungs. Confluent lesiosn in upper lung fields
26	38	61.0	168.0	+	+	0	+	0	1 year	Negative	Fibrosis Emphysema	Increased linear markings. Increased radiability right upper.
27	56	72.2	158.0	+	+++	Iron moulder 7 years	+++	++	1 year	Breath sounds harsh. No râles	Fibrosis	Mottling and feathering throughout both lungs.
28	57	86.2	171.0	0	++ 6 months	Moulder (1896–1917)	+++	+	12 years "tired feeling"	Negative	Fibrosis	Fine feathering throughout both lungs.
29	65	68.8	171.5	+	+	Sand, Stone cutter for 10 years	+	0	2 years	Negative	Fibrosis	Infiltration of right lung. Fine mottling throughout.
30	32	50.2	165.0	+	+	Sand blasting 7 months	0	0	3 months	Scattered râles	Fibrosis	Diffusely accentuated lung markings.

of symptoms varied from 3 months to 12 years. The duration of exposure to silica in the patients suspected of having pneumonokoniosis varied from 7 months to 25 years. One patient (Case 28) complained of muscular pains and dizziness. Cyanosis was observed in half and clubbing of the fingers in 3 of the 14 cases. The blood pressure was within normal limits except in Cases 19 and 30 where it was 140/100 and 160/105 respectively. Electrocardiograms showed left ventricular preponderance in Cases 17, 18 and 26 while a preponderance of the right ventricle was found in only one (Case 17). The spleen was not felt in any of the subjects.

Results

Emphysema. In Table VI are presented the individual and the mean values for the total volume and the volume per kilogram of body weight for the blood, plasma, cells and circulating hemoglobin. The total *blood volume* varied between 3.18 and 7.27 liters with a mean value of 4.87 liters. The mean value of the blood volume per kilogram of body weight was 76.7 cc., a figure somewhat below that of the normal subjects. The extremes were 68.4 and 91.6 cc. All the observations fell within normal limits as shown in Figure 1. The mean value of the *plasma volume* per kilogram was slightly below that of the normal, 40.7 cc. as compared with 42.8 cc. On the other hand the mean value of *cell volume* per kilogram in the pathological cases was 35.5 cc., which was approximately the same as that of the normal group. Only two observations were slightly outside the normal limits as shown in Figure 1. The mean value for the *hemoglobin per kilogram of body weight* was 12.5, slightly below the normal; with 2 cases beyond the lower limits of normal. (Figure 2.) The *hemoglobin per 100 cc. of blood* varied between 11.6 and 18.7 grams with a mean of 16.2 grams, which is identical with that of the normal. The *hematocrit* readings of cell volume also varied markedly between 38.5 to 52.6 per cent with a mean value of 46.3 per cent. We find then, in this group of cases with emphysema, a slight though not significant decrease in the blood and plasma volume and the hemoglobin per kilogram of body weight, while the cell volume remains the same.

Fibrosis. The results in a study of 14 cases of chronic pulmonary fibrosis are tabulated in Table V. In this group we find a definite increase in the mean values of *blood* and *cell volume* per kilogram of body weight, the former mean value being 88 cc. as compared with 79.1 cc. of the normal group; while the latter value was 45.2 cc. compared with the normal value of 35.8 cc. It is evident from Table VI and Figure 1 that the mean values of the whole group are high due to marked increase in the volume of the blood and cells in three (Cases 19, 27 and 28) of the fourteen cases while in the remaining ones the values obtained were within normal limits. The mean value for the *plasma volume* was 42.4 cc., approximately that of the

TABLE VI

Results of the determination of blood volume in 30 cases of chronic pulmonary diseases

Case	Total blood volume liters	Blood/kgm. cc.	Total plasma volume liters	Plasma/kgm. cc.	Total cell volume liters	Cell volume/kgm. cc.	Total hemoglobin grams	Hemoglobin/kgm. grams	Hemoglobin/100 cc. grams	Hematocrit per cent
Mean and standard deviation of 25 normal subjects										
	5.93 ±.77*	79.1 ±7.19	3.22 ±.42	42.8 ±4.4	2.69 ±.39	35.8 ±3.62	982 ±149.9	12.94 ±1.47	16.2 ±.84	45.1 ±2.29
Pulmonary emphysema										
1	3.90	73.5	2.20	41.4	1.76	33.2	610	12.1	16.4	45.2
2	5.30	86.2	2.90	47.1	2.37	38.5	949	15.5	17.9	44.7
3	4.99	85.4	2.78	47.6	2.16	36.9	886	15.2	17.8	43.2
4	3.76	69.6	1.79	33.1	1.95	36.1	648	12.0	17.2	51.7
5	3.80	76.5	1.76	35.4	2.00	40.2	709	14.3	18.7	52.6
6	6.12	79.1	3.21	39.5	3.17	39.0	1029	12.7	15.9	49.4
7	3.86	71.0	1.93	35.9	1.91	35.1	685	12.6	17.8	49.4
8	3.18	69.2	1.92	41.8	1.23	26.8	370	8.0	11.6	38.7
9	3.55	83.3	1.96	46.0	1.57	36.9	546	12.8	15.4	44.1
10	7.27	91.6	3.68	46.3	3.54	44.6	1181	14.9	16.2	48.7
11	6.03	72.5	3.55	42.7	2.44	29.3	865	10.4	14.4	40.5
12	4.65	69.1	2.69	39.9	1.94	28.8	621	9.2	13.4	41.7
13	5.21	68.4	2.86	37.3	2.34	30.6	876	11.4	16.7	44.7
14	6.85	87.8	3.71	47.6	3.10	39.7	1089	14.0	15.9	45.2
15	4.84	69.7	2.31	33.3	2.49	35.9	833	12.0	17.2	51.5
16	4.27	71.9	2.15	37.7	2.09	36.7	726	12.7	16.9	48.9
Mean standard deviation	4.87 ±1.20*	76.7 ±7.9	2.59 ±.65	40.7 ±5.60	2.25 ±.36	35.5 ±4.70	789 ±213	12.5 ±1.94	16.2 ±1.87	46.3 ±3.59

* Standard deviation.

TABLE VI (continued)

Case	Total blood volume liters	Blood/kgm. cc.	Total plasma volume liters	Plasma/kgm. cc.	Total cell volume liters	Cell volume/kgm. cc.	Total hemoglobin grams	Hemoglobin/kgm. grams	Hemoglobin/100 cc. grams	Hematocrit per cent
Mean and standard deviation of 25 normal subjects										
	5.93 ±.77*	79.1 ± 7.19	3.22 ± .42	42.8 ± 4.4	2.69 ± .39	35.8 ± 3.62	982 ± 149.9	12.94 ± 1.47	16.2 ± .84	45.1 ± 2.29
Pulmonary fibrosis										
17	6.15	91.4	3.47	51.6	2.64	39.2	934	13.9	15.2	42.9
18	5.98	84.4	2.96	41.7	2.99	42.2	1017	14.3	17.0	50.0
19	4.70	96.4	1.92	39.4	2.75	56.8	862	17.6	18.3	58.5
20	5.41	81.0	2.80	41.9	2.57	38.5	861	12.90	15.9	47.4
21	3.99	71.0	2.05	36.5	1.91	34.0	627	11.16	15.7	47.8
22	4.56	62.3	2.39	32.6	2.30	31.4	712	9.73	15.6	41.0
23	4.54	82.5	2.52	45.8	1.99	36.2	676	12.3	14.9	43.8
24	4.57	83.1	2.47	45.0	2.08	37.8	684	12.4	15.0	45.4
25	6.54	86.1	3.20	42.1	3.29	43.3	1102	14.50	16.8	50.3
26	5.64	92.5	3.00	49.2	2.59	42.5	809	13.26	14.3	45.8
27	7.94	108.5	3.29	45.5	4.56	63.1	1580	21.8	19.9	57.4
28	11.72	135.9	3.59	41.6	8.06	93.5	2494	28.9	21.3	68.8
29	5.86	85.1	3.27	47.5	2.59	37.6	921	13.4	15.7	44.2
30	6.42	71.1	3.04	33.7	3.35	37.1	1090	12.1	17.0	52.1
Mean standard deviation	6.00 ±1.87*	88.0 ±17.80	2.85 ±.52	42.4 ±5.63	3.12 ±1.51	45.2 ±15.8	1026 ±469	14.9 ±4.82	16.6 ±2.05	49.7 ±7.06
Both groups mean standard deviation	5.3 ±1.65*	82.0 ±14.12	2.70 ±.65	4.18 ±5.4	2.67 ±1.28	39.3 ±12.8	916 ±375	13.33 ±3.60	16.4 ±1.87	47.8 ±5.85
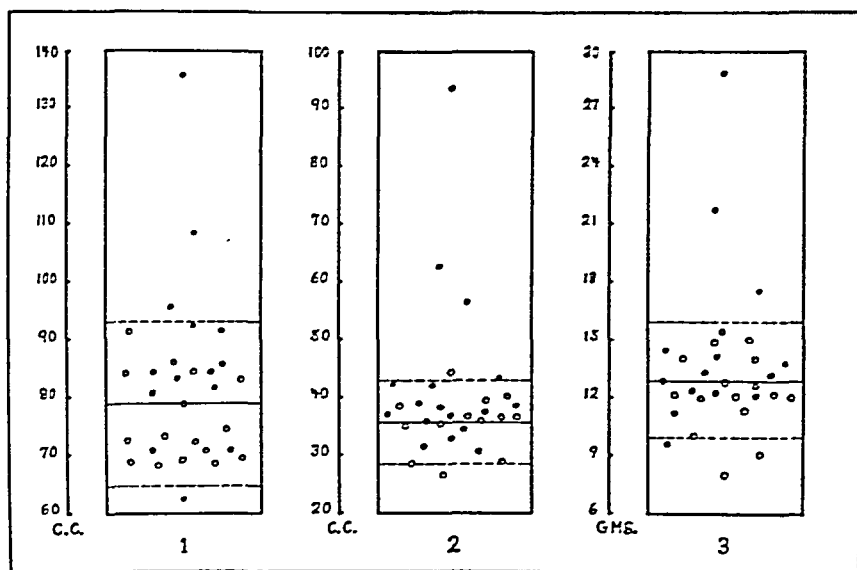
FIG. 1. DETERMINATIONS OF BLOOD AND CELL VOLUME AND HEMOGLOBIN
PER KILOGRAM OF BODY WEIGHT IN 30 CASES OF CHRONIC RESPIRATORY DIS-
EASES.

The mean values for the normal subjects are represented by the unbroken
lines, while the broken ones show the deviations from the mean. Circles indi-
cate cases with emphysema. Dots, those with fibrosis.

1. Blood volume. 2. Cell volume. 3. Hemoglobin.
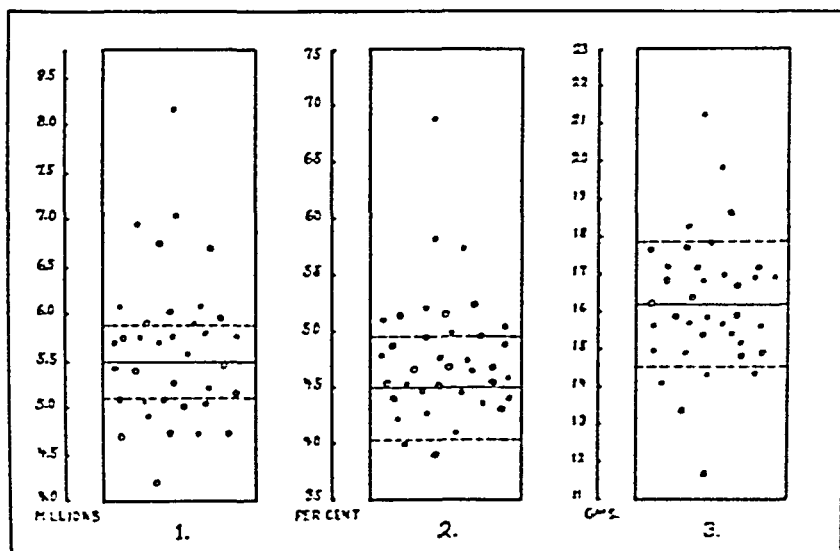
FIG. 2. ERYTHROCYTE COUNT, HEMATOCRIT, AND HEMOGLOBIN PER 100 CC. OF
BLOOD IN 30 CASES OF PULMONARY EMPHYSEMA AND FIBROSIS

1. Red blood cell count per cubic millimeter.
2. Hematocrit.
3. Hemoglobin per 100 cc. of blood.

normal group. Although the values for the *hemoglobin per kilogram of body weight* varied widely, the mean value was 2 grams higher than that of the normal subjects. Here again Cases 19, 27 and 28 were definitely above the normal limits while Case 22 was slightly below, as illustrated in Figure 1. Likewise the mean value of the *hemoglobin per 100 cc. of blood* was somewhat above that of the normal group, while in the same three cases mentioned above the hemoglobin was elevated. Along with the above findings we found the mean for the *hematocrit* somewhat increased, 49.7 per cent as compared with the normal of 45.1 per cent, while 6 of the cases showed an abnormally high value (Figure 2.)

The calculated values based on body weight, and the observed values for the total blood, plasma and cell volume and hemoglobin in the 30 pathological cases are represented in Figure 3. This shows graphically
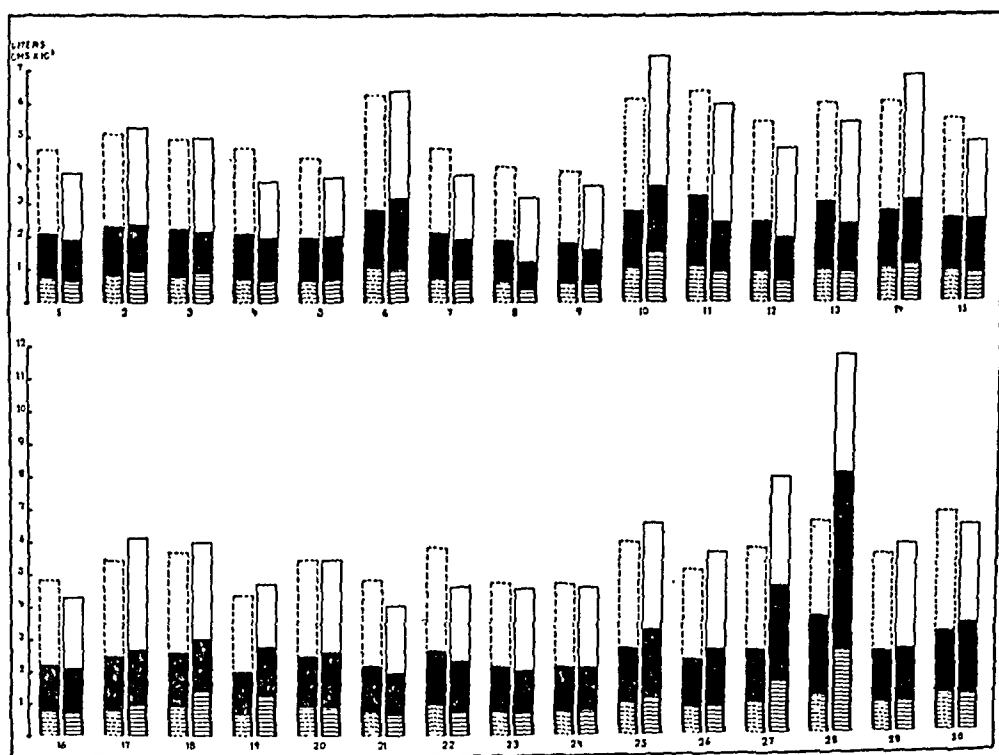
FIG. 3. CALCULATED AND OBSERVED BLOOD VOLUME IN CASES OF
PULMONARY EMPHYSEMA AND FIBROSIS

Cases 1 to 16 inclusive are those with emphysema; from 17 to 30 inclusive, cases of fibrosis. Each case is represented by two columns. The one on the left, with broken lines is the predicted while the right hand column is the observed volume. The white area is plasma; the black, cell volume while the transverse lines represent the total hemoglobin.

that there are no significant variations in cases of pulmonary emphysema (Cases 1 to 17) while in the group with pulmonary fibrosis Cases 27 and

28 show evidence of hydremic plethora [4] and Case 19 shows an increase in the blood and cell volume and hemoglobin while the plasma is slightly reduced.

When the two groups are taken together we find that the mean values for the blood and cell volume, the total hemoglobin and the hematocrit are slightly above those observed in the normal subjects, while the plasma volume is slightly decreased; thus showing slight concentration of the blood.

The mean values for the blood, plasma and cell volume and circulating hemoglobin expressed in terms of cubic centimeters per kilogram of body weight for the three groups (normal, emphysema, and fibrosis) are compared graphically in Figure 4.   A slight decrease in the blood and plasma
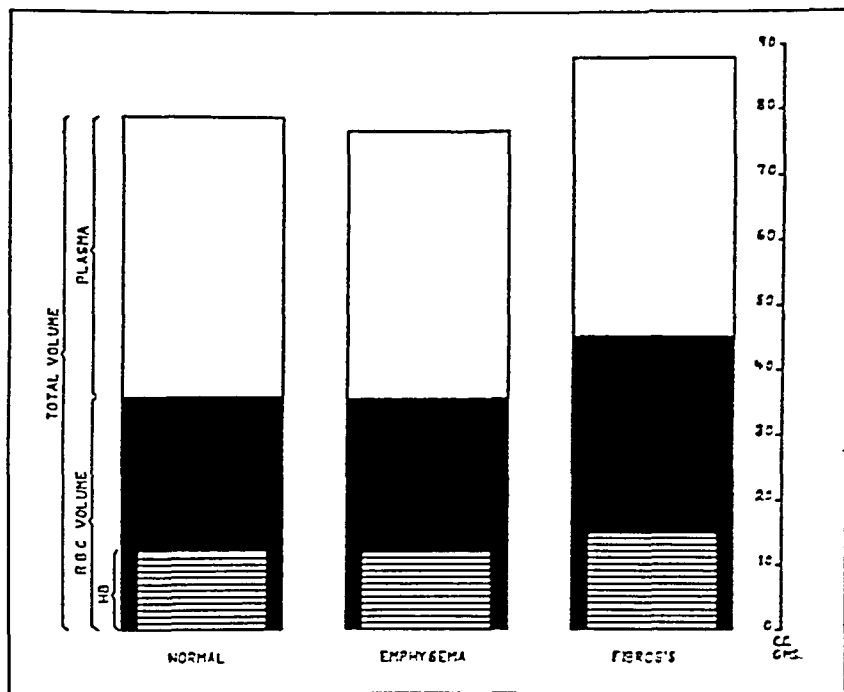
FIG. 4.   MEAN VALUES FOR THE BLOOD VOLUME AND ITS COMPONENTS
PER KILOGRAM OF BODY WEIGHT

volume is evident in the cases of emphysema while the cell volume and hemoglobin are practically identical with the normal group.   On the other hand, the cases with pulmonary fibrosis show an increase in mean values of 11 per cent in the blood volume, of 26 per cent in the cell volume and of 15.5 per cent in hemoglobin as compared with the normal, while no abnormality in the mean plasma volume was observed.

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[4] Same as polycythemic hypervolemia used by Rowntree, designating increase of the volume of blood and cells above the limits for normal individuals.

TABLE VIII

Characteristics of the erythrocytes in 36 cases of pulmonary emphysema and fibrosis

	Normal mean*	Emphysema (18 cases)		Fibrosis (18 cases)		Both groups (36 cases)	
		Mean ± standard deviation	Coefficient of variation	Mean ± standard deviation	Coefficient of variation	Mean ± standard deviation	Coefficient of variation
			per cent		*per cent*		*per cent*
Red blood cells, *millions*............	5.50	5.39 ±0.68†	12.6	5.87 ±0.80†	13.6	5.70 ±0.80†	14.0
Corpuscular volume, *cubic microns*....	82.2	86.8 ±9.95	11.4	83.5 ±6.56	7.8	84.5 ±8.46	10.0
Corpuscular hemoglobin, *grams* $\times 10^{-12}$	28.8	30.1 ±3.16	10.5	27.9 ±2.45	8.7	28.9 ±2.87	9.9
Corpuscular hemoglobin Concentration, *per cent*	34.2	34.7 ±2.64	7.6	33.4 ±2.65	12.6	34.2 ±2.65	7.7

* Mean of normal values in 352 males (18 to 30 years) calculated from Osgood, Wintrobe and Foster.
† Standard deviation.

The blood volumes of 30 of these cases are reported above. The mean value for the number of red cells per c.mm. in both groups was 5.70 millions, a slight increase over that of the normal (5.50 millions) calculated in 352 males reported by Osgood (17), Wintrobe (18), and Foster and Johnson (19). The mean value for the group with emphysema was 5.39 millions a figure slightly below the normal value while that for the group with fibrosis was 5.87 millions, which is definitely above the normal. The extremes in the two groups were 4.2 and 8.17 millions. As illustrated in Figure 2, an equal number of cases fell below as above the limits of normal.

The corpuscular volume for both groups was increased, the mean value being 86.8 μ^3 for the cases showing emphysema and 83.5 μ^3 for the group exhibiting fibrosis, while for the whole group it was 84.5 μ^3 (normal value 82.2 cubic microns).

Correspondingly, the corpuscular hemoglobin for the cases with fibrosis was slightly lower than for those with emphysema, having mean values of 27.9 and 30.1 micromicrograms (gm. $\times 10^{-12}$) respectively. (Normal value 28.8 gm. $\times 10^{-12}$.)

As shown in Table VIII the mean for the corpuscular hemoglobin concentration in both groups was identical with that of the normal. The mean value in cases with emphysema was slightly above, while in the group with fibrosis it was just below the normal value.

TABLE IX

Relation of the number of erythrocytes to their size and hemoglobin concentration

Number of erythrocytes	Cases	Hemoglobin per 100 cc.	Corpuscular volume	Corpuscular hemoglobin	Corpuscular hemoglobin concentration	Percentage of red blood cells hematocrit
million		*grams*	*cubic microns*	*grams × 10⁻¹²*	*per cent*	*per cent*
4.5	5	15.1	93.3	30.4	32.4	44.5
5.0	11	16.2	88.8	31.0	34.9	46.3
5.5	11	15.7	81.2	27.3	33.6	46.8
6.0	3	16.8	81.4	28.0	34.5	49.1
6.5	3	18.3	74.3	26.8	36.4	50.7

Table IX shows the variation of the cellular characteristics, hemoglobin per 100 cc. of blood, and the hematocrit in these cases according to the red cell count. Here was found the inverse correlation of the corpuscular hemoglobin and volume to the red cell count, such as occurs in normal subjects; in other words as the red cell count increased the cells became smaller and contained less hemoglobin; both of these changes being proportional, the corpuscular hemoglobin concentration remained the same.

DISCUSSION

Investigations of the blood volume in cases of chronic pulmonary disease are important from the standpoint of the various theories advanced to explain the production of polycythemia and its relation to a low oxygen tension. The development of these ideas is closely correlated with and based upon observations made at high altitudes, or in chambers where the barometric pressure is lowered to simulate the effects of higher levels. A brief review of the results obtained by such investigations will be useful in understanding the present status of the pathogenesis of polycythemia.

Since the discovery by Paul Bert (20) in 1882 (confirmed a few years later by Viault (21)), that the blood of animals living in the Peruvian Andes has an increased number of red blood cells and an increased capacity for combining with oxygen, practically all investigators agree that such a change occurs both in animals and man at low barometric pressure, although its intensity varies markedly in different individuals. Miescher (22) in 1893 formulated the theory that oxygen deficiency in the bone marrow was the stimulus for the formation of new red cells. His idea has had general acceptance and was substantiated indirectly both by the studies of Bence (23) who claimed that inhalation of oxygen decreases the erythrocytosis which one encounters at high altitudes, and by the observations of Dersca and Valter (24) who succeeded in decreasing the number of red cells in the peripheral blood of patients with anoxemia by the intravenous injection of oxygen. The work of Barcroft and his co-workers (25) demonstrating that the spleen is a storehouse for red cells which are liberated and thrown into the circulation in conditions of oxygen want, has thrown new light on the explanation of erythrocytosis at high altitudes. At the present time most observers agree that this increase in red cells and hemoglobin is primarily produced by the contraction of the spleen, liberating the red cells stored in its pulp, and possibly also by releasing the supply of red cells stored in the bone marrow (McNee (26)). Secondarily, if the condition of oxygen want persists, signs of bone marrow activity appear and the increase in the number of red cells may then be correlated with the signs of overactivity of the hematopoietic system. However, because so few estimations of blood volume have been made, it is still a debated point whether this erythrocytosis in new comers and residents at a high altitude is associated with a real increase in the total volume of blood cells or with changes in their concentration in the blood. In 1910 Douglas (27) made observations by means of the carbon monoxide method on Teneriffe (altitude 7,000 feet) and was unable to detect any changes. The Pike's Peak Expedition (by Haldane and others (28)) found evidence of a temporary concentration of red blood cells during the first few days and later a true increase in the total volume of cells. Smith, Belt, Arnold and Carrier (29) found minimal changes in the blood volume

after a brief stay at Long Lake in the Sierra Nevada Mountains (altitude 11,000 feet), and Lippmann (30) and Laquer (31) found very slight alterations at Davos (5,100 feet). Monge (32) has described the existence of a primary polycythemia in the residents of the Peruvian Andes, but studies of the blood volume have not been made in such cases. It is evident from this review that our knowledge concerning the true nature of the erythrocytosis that man develops at high altitude is insufficient. Whether or not there is an increase in the blood volume similar to that found in cases of polycythemia vera at sea level is not known. Further studies in this connection will undoubtedly help solve this interesting problem.

Harrop and Heath (33), who called attention to the marked resemblance of the symptoms of " mountain sickness " to those found in cases of polycythemia vera, advanced the opinion that polycythemia vera may be due to lowered oxygen tension of the arterial blood caused by a change in the alveolar wall, resulting in decreased oxygen diffusion. In three cases of polycythemia they observed a lowering of the arterial oxygen saturation after exercise. The results of these observations would naturally suggest that polycythemia should be found more frequently in cases of chronic pulmonary diseases, in which pathological alterations in the lung parenchyma exist producing a decrease in the oxygen saturation of the arterial blood. That such is not commonly the case is a well known clinical fact; however, actual studies of the blood volume have been reported in only a few cases. Plesch (14) and Uhlenbruck (5) found an increase of the blood volume in a small number of cases with chronic bronchitis and emphysema, but the degree of anoxemia was not determined. Lemon (15), who studied 12 cases of chronic pulmonary emphysema, did not find any significant increase in the blood and cell volume, in spite of a low value (average 88 per cent) of the arterial oxygen saturation in seven of his cases. From these studies Lemon concluded that there is no relationship between the development of polycythemia and pathological alterations in the alveolar wall. It is clear that the evidence against the relationship between development of polycythemia and pulmonary changes resulting in a decreased oxygen tension is rather negative in character and inconclusive in view of the limited number of cases studied.

A point frequently lost sight of is that the response of the bone marrow to any stimulus calling for the formation of new red cells is extremely variable from individual to individual. This fact is easily demonstrated by the response of the blood to low barometric pressures. Although subjects are exposed to exactly the same stimulus, some will exhibit a great increase in the circulating hemoglobin and red cells, while others will show only a slight increase. This difference is also found among the residents at high altitude (Hurtado (34)). One can not dismiss the hypothesis that

a case of polycythemia vera represents a pathological response of a very sensitive bone marrow to a slight or moderate decrease in the oxygen tension of the arterial blood, and that the background of such an occurrence is the existence of anatomical alterations in the lung parenchyma. In a large majority of cases such alterations may not be sufficient to cause such an abnormal response on the part of the hematopoietic system. It is significant in this connection that in most cases of polycythemia vera in which the arterial blood has been studied, the oxygen saturation was found to be lower than normal.

That we found a hydremic plethora in three cases of pulmonary fibrosis is quite significant and indicates that perhaps these two conditions will be found to be more frequently associated if a greater number of cases are studied. It is also significant that in these three cases the arterial oxygen saturation was markedly decreased. It is fair to assume from the history that in these cases the fibrosis of the lung resulting from inhalation of silica probably preceded the development of the polycythemia. In two of these cases the signs and symptoms with the exception of an enlarged spleen were identical with those of so-called " primary " polycythemia or polycythemia vera. These results indicate that, whereas in most cases of chronic pulmonary diseases there is no significant change in the volume of the blood except possibly slight concentration even though anoxemia exists, there are, however, occasional cases in which a hydremic plethora develops closely simulating polycythemia vera. It is interesting that this change was found only in cases with pulmonary fibrosis. Our cases with emphysema, as well as those reported by Lemon (15), in spite of the existence of anoxemia showed normal blood findings. This cannot be explained in the light of our present knowledge. Whether or not the nature of the pathological changes in the lungs has some relation to the development of polycythemia is unknown.

SUMMARY AND CONCLUSIONS

Determinations of blood and plasma volumes were made on 25 normal male subjects whose average age was 26 years. Intravenous injection of brilliant vital red was used in the estimation of the plasma volume. Studies were made also in 16 cases of pulmonary emphysema and in 14 cases of pulmonary fibrosis. The determinations were made in conjunction with clinical observations and measurements of the pulmonary capacity and of the gas content of the arterial blood. From the results of this investigation the following conclusions are drawn:

1. In a group of normal males there were marked variations in the values for the total blood volume and its components. There was a high and significant correlation between the volume of the blood and body weight and on this basis the expected values for the blood volume in the pathological cases were calculated.

2. In a group of cases with varying degrees of pulmonary emphysema, no significant alterations from the normal were found in the plasma, blood and cell volume or in the total circulating hemoglobin per kilogram of body weight.

3. The mean values for the cell volume and total hemoglobin were definitely increased in the group of cases with pulmonary fibrosis. This was due to marked alterations found in three cases, and not to a consistent increase in all the subjects. The volume of the plasma was normal.

4. No definite correlation was found between the severity and duration of symptoms and the increase in the blood volume.

5. A decreased arterial oxygen saturation was found in the three cases which showed marked alterations in the blood volume, but in the remaining cases no correlation was demonstrated between the degree of anoxemia and the blood findings.

6. Likewise no definite relationship was found between alterations in the blood volume and changes in the pulmonary capacity.

7. The mean values for the grams of hemoglobin and the red cell volume per 100 cc. of blood were within normal limits in the group of cases with pulmonary emphysema, but there was a moderate increase in those with pulmonary fibrosis. Marked individual variations were observed.

8. The mean value for the red cell count was found to be within normal limits in both groups of cases, those with pulmonary emphysema and those with fibrosis. No significant changes were demonstrated in the volume or hemoglobin content of the red blood cells.

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normal subjects, have been fully presented in previous papers. (1), (2). For convenience we may briefly summarize the usual procedures. After a previous rest and with the subject in the recumbent position the residual air was determined by the oxygen dilution method of Christie (3); the vital capacity, reserve and complementary air were graphically recorded. Two observations were made in each case. The external chest measurements were taken, and immediately afterwards with the subject in the same recumbent position a doubly exposed roentgenogram of the chest was obtained at the respiratory positions of forced expiration and inspiration. Measurements were made on this film to determine the degree of chest expansion, and by means of a planimeter the areas of the lung fields at maximum expiration and inspiration. The latter area multiplied by the anteroposterior diameter of the chest in the same respiratory position gave the so-called " radiological chest volume," from which the corresponding normal pulmonary capacity was predicted in each case by means of a regression formula. In each case the observed volumes were compared with the predicted values.

It was anticipated that difficulty would be encountered in establishing a complete admixture between the oxygen-air mixture in the spirometer and the large volume of residual air in the lungs during the rebreathing period in cases of pronounced emphysema. However, we have found that this is not the case. Repeated determinations of the residual air on the same patients have agreed closely, not only during the same day, but also in later observations, and it is quite unlikely that the same degree of imperfect mixing would occur each time. We have concluded that the oxygen dilution method of Christie is applicable to the study of cases of this nature, but as in previous studies (4) we have found it to be more convenient to determine directly the residual air rather than the mid capacity (called " functional residual air " by Christie). It is important that the time of rebreathing be kept constant in all cases, because prolongation of this time may lead to a rise in the nitrogen percentage in the spirometer-lung system due to the continuous excretion of nitrogen from the circulating blood into the alveoli, thus giving an error in the value for the residual volume. Failure to appreciate and correct for this error might lead one to the conclusion that imperfect mixing had occurred. In each of our cases we have employed a rebreathing period of seven minutes.

A summary of the important clinical findings in each case is given in an appendix at the end of this paper. A total of twenty-six cases has been studied, of which twenty-four were males and two females. The ages varied between 17 and 64 years with an average of 45 years; with the exception of two individuals, all were above 20 years of age. Most of the patients had bronchitic asthma, and had signs of emphysema or the complaint of constant shortness of breath. It is obvious that the present study has no statistical relation to the incidence of pulmonary emphysema

in patients with chronic asthma, as the cases were selected on the above basis.

In all cases a complete history and physical examination were recorded, including roentgenographic studies of the chest. In most cases an electrocardiogram was made. The usual complaints were dyspnea and cough, the duration of which varied between one and forty years. Dyspnea was present in all cases. The patient's statement and the physical examination were carefully analyzed in an attempt to estimate the degree of dyspnea, which varied from a sensation of discomfort of which the patient was barely conscious during severe physical activity to marked shortness of breath occurring even at rest. A history of asthmatic attacks at frequent or infrequent intervals was given in all but three instances (Cases 1, 4, and 26), and chronic bronchitis with periods of exacerbations was a complaint common to all the patients. In no case was the determination of the pulmonary capacity or the roentgenological examination of the chest made during the acute respiratory distress of an asthmatic attack.

There was a wide range in the type of chest observed in these patients ranging from the typical barrel shaped emphysematous thorax to those in which the chest appeared of normal size and contour. Chest examination revealed in most cases an increased anteroposterior diameter, limited expansion, low position of the diaphragm, hyperresonance, prolongation of the expiration and scattered râles chiefly of the musical variety. In five cases clubbing of the fingers was present and in eight patients cyanosis of the lips and other mucous membranes was pronounced. The roentgenographic appearance of the lungs varied markedly. Among the most constant and prominent findings were increased linear markings, accentuation of the hilar shadows, increased radiability of the lung fields, flaring of the ribs with a widening of the intercostal spaces; in a few cases there was evidence of pleural thickening, and more infrequently of pleural adhesions about the diaphragm (see Figure 1). In one case the roentgenogram showed an enlargement of the left ventricle and in another a prominent pulmonary conus.

In sixteen cases electrocardiograms were taken. Five of them were interpreted as normal, and in six cases there was evidence of left ventricular preponderance. Myocardial damage was suspected in four cases and sino-auricular tachycardia was observed in two others. One patient showed an intraventricular conduction defect. In no case was there evidence of right ventricular preponderance. None of the cases had peripheral edema or revealed other signs of congestive heart failure. Although the symptoms of marked emphysema and those of cardiac decompensation are somewhat similar, we concluded after a careful consideration of the clinical, radiological and electrocardiographic studies that the cardiac factor played no prominent part in these cases.
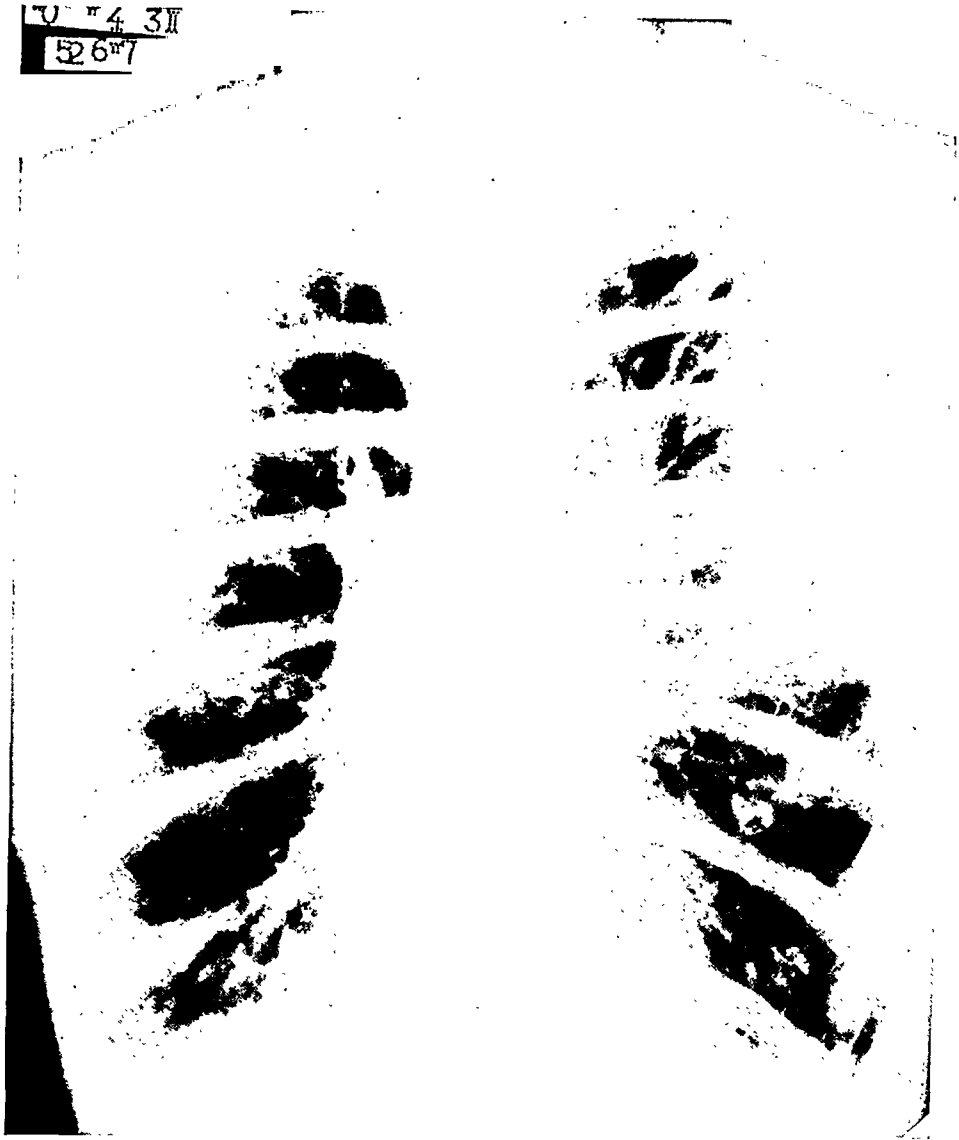
FIG. 1. TYPICAL ROENTGENOGRAPHIC APPEARANCE OF THE LUNGS IN
PULMONARY EMPHYSEMA (CASE 17)

In Case 1 an autopsy revealed changes in the lungs characteristic of
marked emphysema. Grossly they were very voluminous and beneath
the pleural surface several large dilated alveoli were noted, the largest
measuring 5 mm. The cut surface was also emphysematous. A few
yellow raised plaques were visible on the intimal surface of the pulmonary
vessels. Microscopically, the bronchiolar epithelium was everywhere hy-
perplastic and the bronchioles in general were slightly dilated. The epi-
thelium lining some of the markedly dilated alveoli was cuboidal. The
connective tissue stroma, especially about the bronchioles was greatly in-
creased. It was in the portion of this connective tissue adjacent to the
alveoli as well as in the bronchial walls that cellular infiltration was most

marked. Both sides of the heart were somewhat hypertrophied and dilated, most markedly on the right. There was some calcification of the aortic valves. These marked abnormalities of the lungs correlate well with the clinical condition of the patient, the extreme respiratory disability and the marked alterations which were found in the various components of the pulmonary capacity.

The venous blood pressure (indirect method of Eyster (5)) was determined in six cases. The readings varied between 50 and 120 mm. of water, with an average value of 73, which is in the upper zone of normal range. All of the values except one were above 70 mm. of water. These results are similar to those found by Kountz, Pearson and Koenig (20) in 5 cases of pulmonary emphysema.

Other measurements included blood volume, oxygen saturation of the arterial blood, etc. These observations will be presented in other communications and it is sufficient to state here that no significant abnormality was found in the erythrocytes or in the volume of the blood, but a definite decrease in the oxygen saturation of the arterial blood was frequently observed.

Determinations of pulmonary capacity

We have summarized in Table I the observed values for the total pulmonary capacity and its main subdivisions as compared with the corresponding normal values predicted on the basis of the "radiological chest volume." Such comparison may be better appreciated in Figure 2. In the group of patients with emphysema the mean observed value for the vital capacity was 2.88 liters or a decrease of 38.8 per cent of the calculated value, with variations between 1.44 and 4.18 liters. The residual air was greatly increased; the average value was 2.84 liters or an increase of 110.3 per cent over that of the normal, with marked fluctuations between 1.39 and 5.82 liters, while the observed value for the total capacity showed a decrease of only 5.7 per cent of the normal value. It is evident that there was a wide range of variation in these observed volumes, ranging from rather moderate to marked deviations from the predicted capacities. But a close analysis reveals that there were certain constant findings in all cases chiefly characterized by a definite decrease in the vital capacity with a corresponding increase in the residual air, so that the total capacity as a rule approximated the normal value. Of the two components of the vital capacity the complementary air seemed to be the one most affected by the reduction. The mid capacity was also increased in all cases.

We must call attention to the fact that the normal values used for comparison in this series are based on observations in younger adults, and it is likely that the observed deviations from normality would not have been so great if normal subjects of similar ages had been used for comparison. That the vital capacity decreases with age is a well known fact,

TABLE I

Comparison of the calculated and observed values for the pulmonary capacity in emphysema

Case number	Total capacity			Vital capacity			Mid capacity			Residual air		
	Calculated	Observed	Difference	Calculated	Observed	Difference	Calculated	Observed	Difference	Calculated	Observed	Difference
	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent
1	5.98	5.04	−15.7	4.67	1.44	−69.1	2.27	4.08	+79.7	1.31	3.60	+174.8
2	6.43	5.55	−13.7	5.02	3.50	−30.2	2.44	2.67	+9.4	1.41	2.05	+45.4
3	7.25	8.52	+17.5	5.66	2.70	−52.3	2.76	6.56	+137.8	1.59	5.82	+266.0
4	6.47	6.18	−4.5	5.05	4.00	−20.8	2.46	3.56	+44.7	1.42	2.18	+53.5
5	6.68	6.61	−1.0	5.21	3.56	−31.6	2.54	4.33	+70.4	1.47	3.15	+114.2
6	5.87	5.28	−10.0	4.58	2.78	−39.3	2.23	3.48	+55.1	1.29	2.50	+93.9
7	5.03	5.76	+14.5	3.92	3.64	−7.1	1.91	2.96	+54.9	1.11	2.12	+90.9
8	6.60	5.40	−18.2	5.15	3.18	−38.2	2.51	3.10	+23.5	1.45	2.28	+57.2
9	5.88	6.46	+9.8	4.59	2.08	−54.8	2.23	5.34	+139.4	1.29	4.38	+239.5
10	6.15	7.20	+17.0	4.80	3.24	−32.5	2.34	4.80	+105.1	1.35	3.96	+193.3
11	7.02	5.23	−25.5	5.48	3.00	−45.2	2.67	3.25	+21.7	1.54	2.23	+44.8
12	6.32	4.77	−24.5	4.93	1.94	−60.6	2.40	3.81	+58.7	1.39	2.83	+103.6
13	5.64	6.26	+10.9	4.40	1.72	−60.9	2.14	5.36	+150.4	1.24	4.54	+267.7
14	4.78	4.66	−2.5	3.44	2.46	−28.5	2.15	2.90	+34.8	1.34	2.20	+64.1
15	4.32	3.74	−13.4	3.37	2.02	−40.0	1.64	2.64	+37.9	0.95	1.72	+91.5
16	6.01	6.58	+9.4	4.69	3.92	−16.4	2.28	3.64	+59.6	1.32	2.66	+101.5
17	8.54	6.64	−22.2	6.66	3.05	−54.2	3.25	4.67	+43.7	1.88	3.59	+90.9
18	6.60	6.09	−7.7	5.15	3.76	−26.9	2.51	3.36	+33.8	1.45	2.33	+60.7
19	3.82	4.20	+9.9	2.75	2.48	−9.8	1.72	2.40	+39.5	1.07	1.72	+60.7
20	7.05	6.75	−4.2	5.50	2.92	−46.9	2.68	4.33	+61.5	1.55	3.83	+147.1
21	5.28	4.30	−18.5	4.12	2.30	−44.1	2.00	2.32	+16.0	1.16	2.00	+72.4
22	4.16	3.65	−12.2	3.25	2.26	−30.4	1.58	1.85	+17.1	0.91	1.39	+52.7
23	6.14	5.77	−6.0	4.79	3.52	−26.5	2.23	3.09	+38.5	1.35	2.25	+66.6
24	5.56	6.05	+9.8	4.34	4.18	−3.7	2.11	2.87	+36.0	1.22	1.87	+53.3
25	6.52	6.21	−4.7	5.09	2.34	−54.0	2.48	4.67	+88.3	1.43	3.87	+170.6
26	6.73	5.49	−18.4	5.25	2.34	−55.4	2.56	3.51	+37.1	1.48	3.15	+112.8
Mean and probable error	6.08 ± 0.14	5.73 ± 0.14	(− 5.7) %	4.71 ± 0.11	2.88 ± 0.13	(−38.8) %	2.31 ± 0.04	3.68 ± 0.14	(+59.3) %	1.35 ± 0.02	2.84 ± 0.14	(+110.3) %
Standard deviation	1.05	1.06		0.87	1.04		0.34	1.08		0.19	1.06	
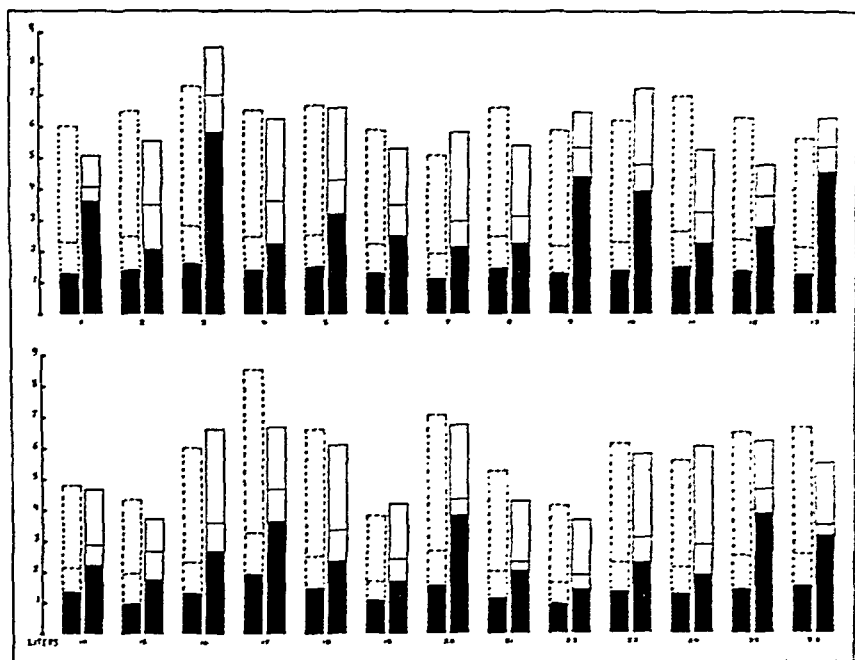
FIG. 2. CALCULATED AND OBSERVED PULMONARY CAPACITY IN EMPHYSEMA

Each case is represented by two columns: on the left with broken lines the calculated value is given; on the right is the observed pulmonary capacity. The black area represents the residual air; the white space above is the vital capacity. The line dividing the vital capacity is the level of the mid capacity.

although according to several investigators (6) (7) (8) (9) a significant lowering is not evident until after 60 years of age. Whether this is accompanied by a corresponding increase in the residual air is not known and an investigation of this matter is now being made in this clinic. However, the changes are so constant and well marked that we do not believe that the general conclusions will be altered. It may be mentioned here that in the cases of the two female patients the normal values found for this sex (4) have been used for comparative purposes.

The observed relative values (total capacity = 100 per cent) of the main subdivisions of the total pulmonary capacity are presented in Table II and graphically in Figure 3. One may readily see that there is a marked decrease in the relative value of the vital capacity with a corresponding increase in the residual air. The mid capacity is also increased. The significance of these findings in relation to the respiratory disability will be discussed later. Also the complementary air makes up a smaller percentage of the vital capacity (mean value 70.8 per cent) than in normal subjects (79.4 per cent). In some cases the reduction in the complementary

TABLE II

Relative values for the subdivisions of pulmonary capacity in emphysema

Case number	Ratio Vital capacity / Total capacity × 100	Ratio Mid capacity / Total capacity × 100	Ratio Residual air / Total capacity × 100	Ratio Complementary air / Vital capacity × 100
1	28.6	80.9	71.4	66.6
2	63.1	48.1	36.9	82.3
3	31.7	77.0	68.3	72.6
4	64.7	57.6	35.3	65.5
5	53.8	65.5	46.4	66.8
6	52.7	65.9	47.3	64.7
7	63.2	51.4	36.8	77.0
8	57.8	57.4	42.2	74.2
9	32.2	82.6	67.8	53.8
10	45.0	66.6	55.0	74.1
11	52.3	62.1	42.7	66.0
12	40.7	79.8	59.3	49.4
13	27.5	85.6	72.5	52.3
14	52.8	62.2	47.2	75.6
15	54.1	70.6	45.9	54.4
16	59.6	55.3	40.4	75.0
17	45.9	70.4	54.1	64.6
18	61.8	48.1	38.2	84.0
19	59.0	57.1	41.0	72.6
20	43.3	64.1	56.7	82.8
21	53.5	53.9	46.5	86.1
22	61.9	50.7	38.1	79.6
23	61.0	53.5	39.0	76.1
24	69.0	47.4	31.0	76.0
25	37.6	75.2	62.4	65.8
26	42.7	63.9	57.3	84.6
Mean and probable error	50.4 ± 1.49	64.0 ± 1.52	49.6 ± 1.52	70.8 ± 1.37
Standard deviation	11.3	11.5	11.5	10.4

air is so great, as compared with that of the reserve volume, that the vital capacity is made up almost equally of these two subdivisions (see Figure 4). Whether this abnormal composition of the vital capacity, that is the relative increase in the reserve air, has a rôle in itself in the respiratory difficulty of the emphysematous patient is an important point to be considered in future studies.

Previous investigations of the pulmonary capacity in cases of emphysema have yielded results identical with those obtained by us. Bruns (10), Bittorf and Forschbach (11) in 1910, Porges, Leimdörfer and Markovici (12), Plesch (13) in 1913, Lundsgaard and Schierbeck (14) in 1923, and more recently Anthony (15), Herms and Rüttgers (16), Hurtado, Fray and McCann (17) and Christie (18) have also reported a relative and absolute increase in the residual air with a proportional reduction of the
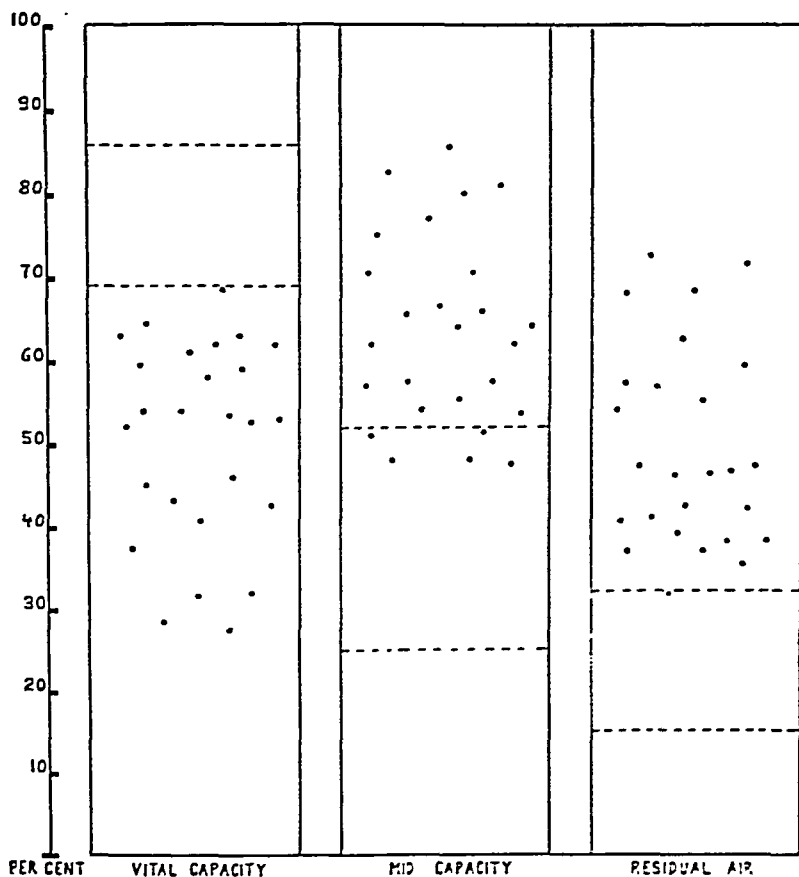
FIG. 3. VITAL CAPACITY, MID CAPACITY AND RESIDUAL AIR EXPRESSED IN
PERCENTAGE OF THE TOTAL CAPACITY

Dots represent individual observations. The areas between lines are the
limits of normal variation.

vital capacity so that the total capacity is kept within normal limits. In a
few cases the latter was found to be higher than the normal due to a great
increase in the residual volume; Cases 3 and 10 in our series belong to this
group.

Christie (18) in his study of seven cases of pulmonary emphysema calls
attention to the fact that the reserve air is constantly decreased or even ab-
sent; an observation not entirely in agreement with our experience in this
larger group of cases. Although the reduction in the reserve air has been
frequently observed it has not usually been great, and even in cases of
marked emphysema with a large increase in the residual air we have ob-
served a nearly normal value of the reserve air, as compared with the con-
siderable decrease in the complementary air. We have been unable to con-
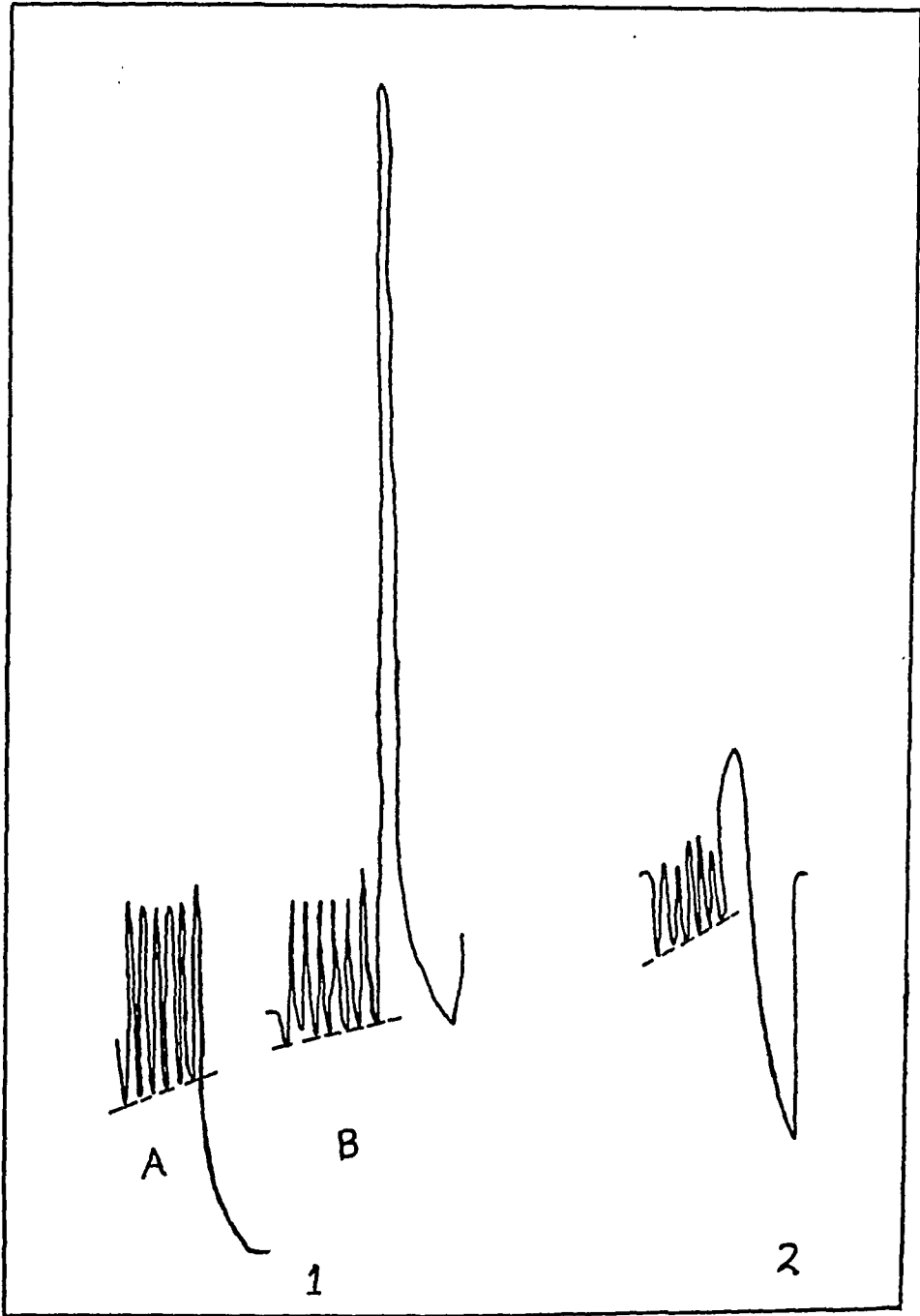
FIG. 4.   RESPIRATORY TRACINGS IN CASES OF PULMONARY EMPHYSEMA

1. *A*—the reserve volume after a tidal inspiration.   *B*—the reserve volume in the same patient after a deep inspiration.   Notice the decrease of this volume and the prolongation of the last part of the expiratory act, i.e. Christie's sign. 2. Relative increase of the reserve air as part of the vital capacity.

firm the observation of Christie that the decrease or disappearance of the reserve air after a deep inspiration, as compared with that determined after a normal tidal inspiration, is a constant finding. He interprets this observation as important evidence of the loss of pulmonary elasticity. In Table III are the data of 22 cases studied from this point of view which show that this phenomenon was found in only nine instances (41 per cent).

TABLE III

Cases of emphysema in which the reserve air was decreased by 100 cc. or more after a forced inspiration *

Case number	Amount of reduc- tion	Vital capacity			Residual air			Ratio $\dfrac{\text{Residual air}}{\text{Total capacity}} \times 100$
		Calcu- lated	Ob- served	Differ- ence	Calcu- lated	Ob- served	Differ- ence	
	liters	*liters*	*liters*	*per cent*	*liters*	*liters*	*per cent*	
2	0.40	5.02	3.50	−30.2	1.41	2.05	+45.4	36.9
6	0.16	4.58	2.78	−39.3	1.29	2.50	+93.9	47.3
8	0.12	5.15	3.18	−38.2	1.45	2.28	+57.2	42.2
10	0.12	4.80	3.24	−32.5	1.35	3.96	+193.3	55.0
14	0.14	3.44	2.46	−28.5	1.34	2.20	+64.1	47.2
15	0.54	3.37	2.02	−40.0	0.95	1.72	+91.5	45.9
16	0.40	4.69	3.92	−16.4	1.32	2.66	+101.5	40.4
18	0.34	5.15	3.76	−26.9	1.45	2.33	+60.7	38.2
24	0.16	4.34	4.18	− 3.7	1.22	1.87	+53.3	31.0

* Of 22 cases investigated only 9 (41 per cent) showed such a decrease.

These data also show no relationship between the decrease in the reserve air and the increase in the residual volume or the decrease in the vital capacity. We do not believe that the failure to find this abnormality is due to any lack of cooperation or training on the part of the subjects, as only those who were intelligent enough to be reliable were selected for this study. In each instance at least four determinations of reserve air were made after normal tidal breathing and three observations of the vital capacity were made. In Figure 4 is shown the respiratory tracing of a patient to which the decrease in the reserve air after a full inspiration was observed. It may also be noted that the curved end of the respiratory tracing is evidence of the difficulty in accomplishing the last part of the expiratory act.

It would be of interest to know whether or not there is a larger respiratory dead space in cases of pulmonary emphysema. An increase in dead space would present a further obstacle to efficient alveolar ventilation. We have investigated this matter in 12 cases, the results of which are presented in Table IV. The data show that the observed values of the dead space vary considerably, being influenced chiefly by the depth of the respiration. This fact has been previously observed in normal subjects (4). The dead

TABLE IV

Respiratory dead space (calculated from the tidal volume and the CO_2 and O_2 percentage of the alveolar and expired air)

Case number	Ventilation per minute	Respirations per minute	Tidal volume	Dead space (from CO_2 per cent)	Dead space (from O_2 per cent)
	liters		*liters*	*cc.*	*cc.*
2	6.75	18	0.35	132	122
5	7.12	6	1.31	496	660
6	8.86	18	0.48	216	221
9	8.49	21	0.40	232	193
11	6.89	18	0.38	197	221
14	6.45	16	0.40	159	176
15	5.40	23	0.24	47	17(?)
16	7.11	16	0.45	153	184
19	7.23	15	0.47	182	181
20	8.44	17	0.51	91	83
23	9.18	19	0.48	166	148
25	7.04	16	0.44	204	229
Average	7.41	17	0.49	189	203

space of emphysematous subjects, when calculated on the basis of the tidal volume and the carbon dioxide and oxygen percentages of the alveolar and expired air, was found to average 189 and 203 cc. respectively in observations in which the average tidal volume was 0.49 liter. These values were slightly higher than those usually found in normal subjects (dead space of 150 cc. for a tidal volume of 0.50 liter). However, since all investigators agree that no true sample of mixed alveolar air from subjects with pulmonary emphysema is ever likely to be obtained by the usual procedure, some caution must be used in accepting these results. In view of the questionable character of the calculations employed above we have commenced to use a new procedure for the measurement of the dead space in the hope of achieving more reliable results. The method consists in taking several samples of air in rapid succession during the course of a single expiration. The total volume of the expiration is graphically recorded, and the time and volume which has passed at the instant of taking each sample is shown. In this way the curve of the gradient of CO_2 in a single expiration may be plotted and used as a basis for calculation of the dead space. These investigations are being carried out at the present time. Two of the curves are presented in Figure 5, one obtained in a normal subject and another in a case of emphysema (Case 25). The point at which the carbon dioxide content of the expired air becomes constant is not appreciably different in the curves of these two subjects. Although no definite conclusion has been reached at the present time, it seems likely that the respiratory dead space in emphysema does not differ appreciably from that of the normal subject.
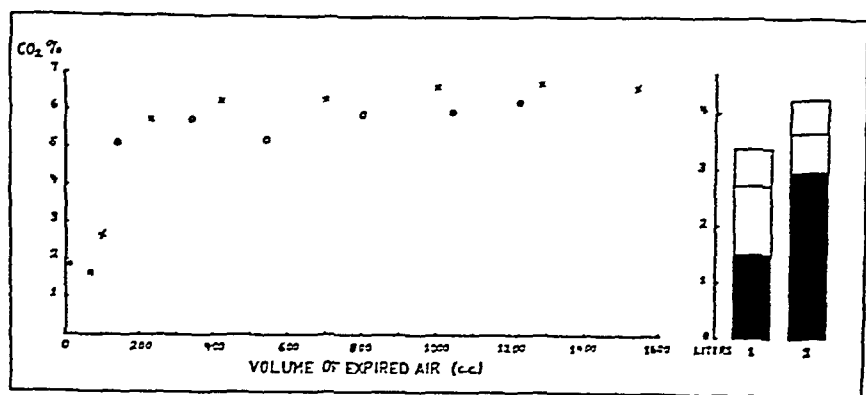
FIG. 5. CO_2 CONTENT OF SEVERAL SAMPLES OF AIR TAKEN AT KNOWN VOLUMES IN A SINGLE EXPIRATION, IN A NORMAL MALE SUBJECT (CROSSES) AND IN A CASE OF PULMONARY EMPHYSEMA (CIRCLES).

Columns on the right represent the total air content of the lungs at the moment the expiration was started: black area represents the residual air; the upper white area is the tidal volume and the lower white area is the reserve air.

Column 1 corresponds to the normal subject and column 2 to the case of emphysema.

Chest expansion

We have studied the expansion of the chest by means of a doubly exposed roentgenographic film taken at the respiratory positions of forced expiration and inspiration with the patient in the recumbent position. Measurements of the areas of the lung fields (by means of a planimeter) and of the excursion of the diaphragm and the lateral expansion of the chest, as well as the degree of rotation of the ribs gave a fairly good indication of the ability and extent to which the thorax may be expanded. Such measurements are summarized in Table V. The ratio,

$$\frac{\text{Area at maximum expiration}}{\text{Area at maximum inspiration}} \times 100,$$

had a mean value of 73.4 per cent with extremes of 56.6 and 90.9. This mean value was distinctly higher than the one obtained in the study of a group of normal subjects (62.2 with a standard deviation of 4.4). All cases, except two, showed a higher ratio than the mean normal value.

A more or less constant finding in this series of cases was a diminution of the diaphragmatic excursion and of the lateral expansion of the chest, the mean values of which were 4.0 and 2.4 centimeters respectively, as compared with the normal mean values of 6.3 and 3.2 centimeters. The degree of rib movement was also decreased in most cases and in some instances there was practically no rotation of the rib during the two phases of respiration. In spite of the wide variation in the measurements above mentioned it is evident that there is a definite diminution in the ability

TABLE V

Chest expansion * in emphysema

Case number	Area at maximum expiration / Area at maximum inspiration × 100	Diaphragmatic excursion			Rib movement	Lateral expansion
		Right	Left	Average		
		cm.	*cm.*	*cm.*	*degrees*	*cm.*
1	82.9	4.1	5.5	4.8	2	0.2
2	74.7	1.0	4.1	2.6	17	3.4
3	86.8	3.4	3.3	3.4	7	2.2
4	70.7	4.8	5.5	5.1	11	1.0
5	71.8	4.1	5.7	4.9	15	3.0
6	73.4	3.6	4.9	4.3	18	2.3
7	75.5	4.3	4.4	4.4	22	2.2
8	71.8	5.2	5.4	5.3	9	1.6
9	80.6	2.3	4.7	3.5	11	1.7
10	82.6	1.7	1.1	1.4	18	3.5
11	71.3	3.9	6.0	5.0	14	2.9
12	85.7	1.9	3.9	2.9	9	0.6
13	84.9	2.1	2.8	2.5	3	0.4
14	66.3	2.9	4.9	3.9	22	1.6
15	68.8	5.2	4.5	4.9	17	2.7
16	69.0	3.3	4.3	3.8	24	3.2
17	90.9	0.0	0.6	0.3	20	2.3
18	64.8	6.0	6.0	6.0	16	2.2
19	68.7	4.5	4.2	4.4	24	3.1
20	67.3	5.4	4.8	5.1	16	1.9
21	56.6	6.3	6.2	6.3	12	2.2
22	68.5	2.0	2.3	2.2	24	3.7
23	61.2	5.5	7.5	6.5	19	3.5
24	59.1	5.5	5.5	5.5	19	3.8
25	75.3	2.4	3.4	2.9	16	2.6
26	85.9	0.5	2.2	1.4	9	2.4
Mean and probable error	73.4 ± 1.20	3.6 ± 0.22	4.4 ± 0.21	4.0 ± 0.21	16 ± 0.79	2.4 ± 0.13
Standard deviation	9.1	1.7	1.6	1.6	6.0	1.0

* Measured from a doubly exposed roentgenogram: at maximum expiration and inspiration.

to expand the chest in pulmonary emphysema, which tends to be proportional to the degree of abnormality in the ratio of residual air to total capacity.

DISCUSSION

The term emphysema is one which is frequently used loosely. Various types of the disorder may be recognized. One form is the *compensatory* emphysema which develops in some portions of the lungs when other portions are rendered airless by any one of a variety of pathological proc-

esses. *"Physiological"* emphysema is found in individuals at high altitudes. The two remaining types may be described as the *obstructive* and *non-obstructive*. The former occurs in association with bronchial obstruction by bronchitis or asthma. The latter is believed to be due to extra pulmonary causes. Kountz and Alexander (19) have studied the non-obstructive type which they believe is due primarily to an increase in the size of the chest resulting from a straightening of the dorsal spine. In this type there is probably little if any loss of respiratory function. We have investigated one such case, the findings of which are presented in Figure 6 for the purpose of comparison with the obstructive type. In this case there was no history of dyspnea or any other symptom referable to the respiratory system, the diagnosis of pulmonary emphysema was made on the suggestive appearance of the chest. Determination of the pulmonary capacity revealed a decreased vital capacity but a normal volume of residual air and a normal ratio of this volume to the total capacity. This case serves to emphasize the importance of differentiating the two types, one of which appears to be chiefly an anatomical abnormality in which respiratory function is slightly disturbed. the other a functional disturbance of great clinical significance. Determinations of pulmonary capacity are helpful in deciding to which group an individual case belongs.

From the clinical history of our patients it will readily be seen that they belong to the *obstructive* type of emphysema. with respiratory disability of varying degree. There was a history of chronic bronchial asthma or bronchitis in all cases. A comparison of this group as a whole with that of normal male subjects studied under similar conditions and with identical technique will be helpful in understanding the different factors which enter into the pathological physiology of pulmonary emphysema. Such comparison is made in Table VI and Figure 7. Patients with emphysema are characterized as a rule by an unusually voluminous chest. by a low position of the diaphragm. The chest in such cases tends to assume a rounded as well as an elongated shape. as though it were permanently in the position of deep inspiration. Such an abnormal chest moves less than that of a normal man and this diminution of expansion was demonstrated by the limited excursion of the diaphragms. by less than normal change in the lateral and antero-posterior diameters of the chest and by the decreased rotation of the ribs. These abnormalities in the mensuration of the thorax are strikingly reflected and paralleled by alterations in the capacities of the lungs. The amount of air which such a patient is able to take into his lungs by a forced inspiration is markedly reduced. and this is due. literally speaking. to the fact that these organs already contain an increased volume of air. In obstructive emphysema the mid capacity at the end of the usual expiration is higher than normal, with the result that each tidal inspiration must diffuse with a larger volume
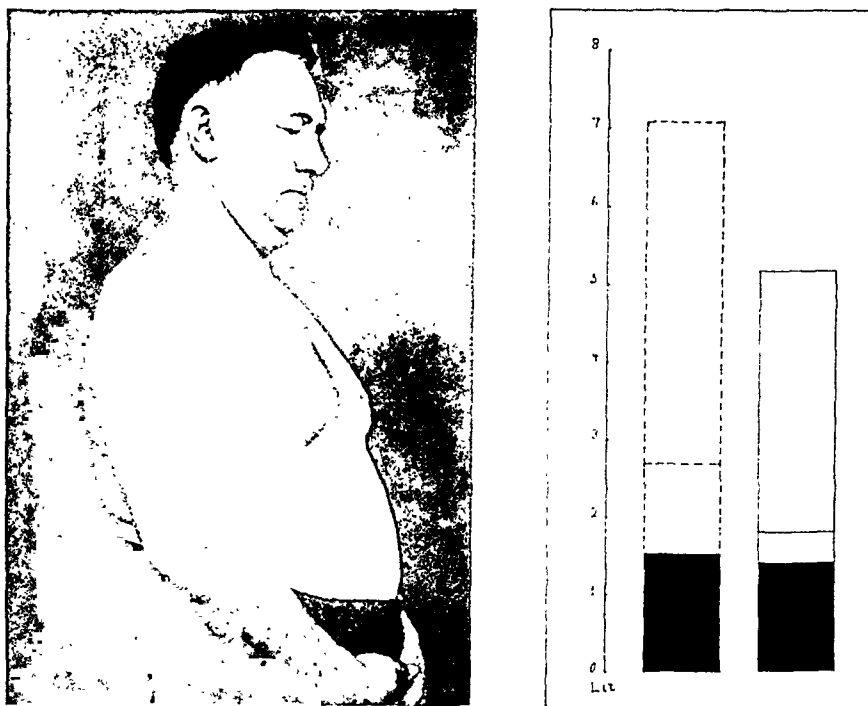
FIG. 6.   PULMONARY CAPACITY IN A CASE OF NON-OBSTRUCTIVE EMPHYSEMA
WITH NO RESPIRATORY DISABILITY

Chest has a definite emphysematous appearance. The vital capacity is decreased but the absolute and relative values of the residual air are normal.
(Ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100 = 27.5$ per cent.)

of air in order to ventilate the alveoli properly. It is apparent that this lack of physiological balance between the volume of air to be ventilated and air available for such ventilation, together with the decreased ability to properly expand the chest, assumes a much greater significance during physical activity when the heightened body metabolism demands an increase in alveolar ventilation.

We may now inquire into the anatomical and functional mechanisms responsible for the abnormalities found in patients with pulmonary emphysema. Although postmortem studies of emphysematous lungs do not always present a uniform picture there is general agreement that certain common findings constitute the anatomical background. When the thorax is opened the lungs appear voluminous and do not collapse. There is usually dilatation and an ischemic appearance of the alveoli chiefly in the peripheral zones. Histologically the alveoli are found to be large and in many places two or more are united in a single cavity due to broken walls. The alveolar walls are usually thinned, stretched, torn and atrophied and the capillaries obliterated. The respiratory bronchioles also present ab-

and his co-workers, which has been recently summarized by Burwell (22). It is of special importance, however, to know the ratio $\frac{\text{Residual air}}{\text{Total capacity}}$ in pulmonary emphysema, in which the residual air is always increased. Our observations indicate that this ratio has great functional significance.

Some investigators have already pointed out the value of such determinations in pulmonary and cardiac diseases and in other conditions. Binger (23) in 1923 found a correlation between the ratio of the vital to the total capacity and the clinical condition of patients with heart disease. Bendove (24) in 1925 observed that in tuberculosis patients with a low vital capacity there was marked dyspnea, while in cases in which a therapeutic pneumothorax had been induced and where the same low vital capacity existed there was no appreciable dyspnea. This difference he attributed to the fact that the residual air had also been reduced so that a more efficient alveolar ventilation was possible. In 1930 Meakins and Christie (25) wrote: "the efficiency of the pulmonary ventilation would appear to rest upon the relationship between the residual air and the total lung capacity." Robb and Weiss (26) in 1932 reported that both in cardiac patients and in those with hyperthyroidism the ratio of residual air to the vital capacity was correlated with the degree of dyspnea present and that this ratio decreased as the condition of the patient improved. We have correlated in Table VII the degree of dyspnea, with the ratio of re-

TABLE VII

Relation of dyspnea to changes in the pulmonary capacity

Dyspnea[*]	Number of cases	Decrease in vital capacity	Increase in residual air	Ratio $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$
		per cent	*per cent*	
+	7	−18.3	+72.0	38.3
++	8	33.8	84.5	43.5
+++	8	54.5	158.1	60.8
++++	1	60.9	267.7	72.5

* Degree of dyspnea indicated by +: dyspnea only on severe physical activity; ++: on moderate exertion; +++: on slight physical activity; and ++++: at rest.

sidual air to total capacity.[2] The degree of dyspnea was estimated from the history and in a few cases from actual observations made during physical activity. A striking relationship between the degree of dyspnea and the value found for the ratio, $\frac{\text{Residual air}}{\text{Total capacity}} \times 100$ is shown in Figure 8.

[2] We have omitted Cases 14 and 26 in this comparative study on account of lack of precise information regarding the degree of dyspnea.
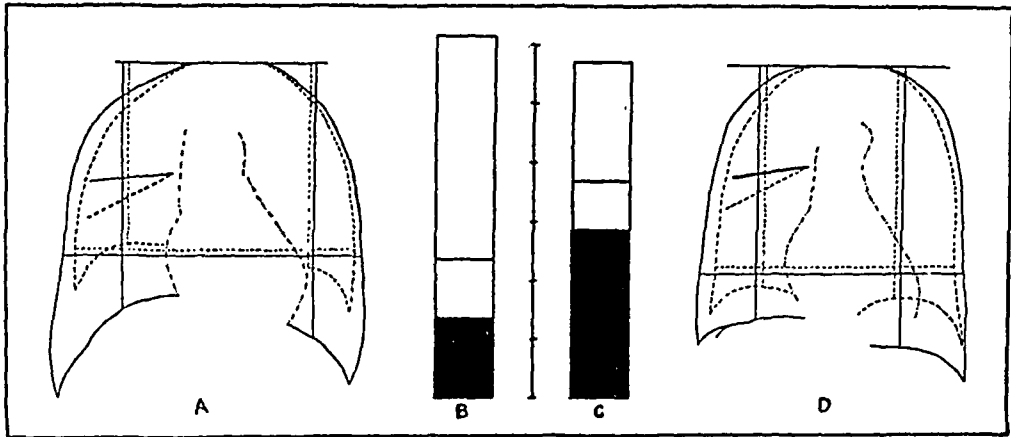
FIG. 7. COMPARISON OF THE AVERAGE CHEST SIZE AND EXPANSION AND THE PULMONARY CAPACITY IN A GROUP OF 26 CASES OF EMPHYSEMA (OBSTRUCTIVE TYPE) WITH THE CORRESPONDING AVERAGE MEASUREMENTS IN A GROUP OF 50 NORMAL MALES. (For actual values see Table VI.)

A. Outline of the lung fields of normal subjects at the respiratory positions of forced expiration and inspiration.

B. Pulmonary capacity in normal subjects.

C. Pulmonary capacity in emphysema.

D. Outline of the lung fields at the respiratory positions of deep expiration and inspiration in emphysema.

normalities in their structure, showing dilatation or evidence of a chronic infective process.

The functional abnormalities of the emphysematous lungs have been discussed recently by Christie (18) in a most interesting and careful study. According to this investigator (to whom we refer the reader for a complete presentation of the related literature) the fundamental factor in the emphysematous lung is a loss of elasticity causing a gradual increase in the residual air, as the lungs yield to the continuously exerted negative pressure at the pleura. The intrapleural pressure increases, shifting to positive values, a fact which has been reported by other investigators (Kountz, Pearson and Koenig (20)). It is obvious from these observations that we are dealing in pulmonary emphysema with a fundamental abnormality in the mechanics of the respiratory process concerned with the proper alveolar ventilation, the importance of which has been admirably summarized by Peabody (21) in the following sentence: " Nevertheless, it remains true that the respiratory process is initiated in the rhythmic expansion and collapse of the lungs and all other phases of respiration depend primarily on the comparatively simple mechanical *movements.*" When we consider the relationship of abnormal pulmonary capacities to the degree of respiratory disability we find in the literature that of all the subdivisions, the vital capacity is the one to which chiefly attention has been paid, especially in the fundamental and classical work of Peabody

and his co-workers, which has been recently summarized by Burwell (22). It is of special importance, however, to know the ratio $\dfrac{\text{Residual air}}{\text{Total capacity}}$ in pulmonary emphysema, in which the residual air is always increased. Our observations indicate that this ratio has great functional significance.

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[3] We have omitted Cases 14 and 26 in this comparative study on account of lack of precise information regarding the degree of dyspnea.

FIG. 8. RELATION OF DYSPNEA TO ⌐¬ RATIO $\dfrac{\text{RESIDUAL AIR}}{\text{TOTAL CAP}}$ ×100

IN PULMON EMA

+   Dyspnea only   ysical activi
++   Dyspnea on m   i
+++   Dyspnea on sl   activity.
++++   Dyspnea at r

them died shortly after the operation and the other had a long and stormy convalescence.   The accuracy of the diagnosis of pulmonary emphysema, as well as the proper estimation of the degree of respiratory disability would be of considerable help in such cases.   In chronic bronchial asthma, also, the development of pulmonary emphysema and the estimation of the degree of respiratory disability is of importance.   In this condition the determination of the ratio of residual air to total pulmonary capacity may have value.   A comparison in such cases of the pulmonary capacity with the duration of the asthma is summarized in Table VIII.   No definite

TABLE VIII

Correlation between duration of asthmatic history and pulmonary capacity

Duration of asthmatic attacks	Number of cases	Decrease in vital capacity	Increase in residual air	$\dfrac{\text{Ratio}}{\dfrac{\text{Residual air}}{\text{Total capacity}}} \times 100$
		per cent	*per cent*	
Less than 5 years..	7	30.2	105.6	44.4
From 5–10 years...	6	42.0	84.8	49.0
From 10–20 years..	5	34.1	92.4	46.8
Over 20 years.....	5	40.1	167.5	55.4

relationship was found between these factors, suggesting that there must be other factors apart from the chronicity of the asthma influencing the development of pulmonary emphysema in these patients.

Finally we wish to call attention to the absence of serious cardiac complications in this group of cases of pulmonary emphysema.   This is in agreement with the investigations of Alexander, Luten and Kountz (28) and Kountz, Alexander and Dowell (29).

SUMMARY AND CONCLUSIONS

Determinations of total pulmonary capacity and its subdivisions and measurements of the capacity to expand the chest have been made in twenty-six cases of pulmonary emphysema of the *obstructive* type.   The findings have been correlated with the degree of respiratory disability, and lead to the following conclusions:

1. The ability to expand the chest in pulmonary emphysema is diminished.

2. There are definite alterations in the subdivisions of the pulmonary capacity consisting chiefly in absolute and relative decrease in the vital capacity, and absolute and relative increase of the mid capacity and residual air.   The total capacity is as a rule normal.

3. The decrease in the vital capacity affects chiefly the complementary air.   Decrease or disappearance of the reserve air after a deep inspiration is not a constant finding in all cases of pulmonary emphysema.

4. Although no definite conclusion has been reached as to the value for the respiratory dead space in pulmonary emphysema it appears likely that no significant alteration from normal is present.

5. The degree of respiratory disability has been found to be closely correlated with the ratio, $\dfrac{\text{Residual air}}{\text{Total capacity}} \times 100$. The higher this ratio the more pronounced is the failure of respiratory adaptation to physical activity.

6. The significance of this ratio as an indication of the efficiency of the alveolar ventilation, and its usefulness in pulmonary emphysema for diagnostic and prognostic purposes has been discussed.

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APPENDIX

Case 1. Male, 61 years. No history of asthmatic attacks. Dyspnea [*] (+++) and cough for 3 years. Marked cyanosis and clubbing of the fingers. Chest emphysematous in appearance with hyperresonant percussion note and a few scattered râles. Electrocardiogram showed myocardial damage and intra-ventricular conduction defect. Roentgenograph: lung markings greatly exaggerated with marked emphysematous changes. Left ventricular hypertrophy.

Case 2. Male, 48 years. Mild asthmatic attacks, dyspnea (++) and cough for 3 years. Chest not emphysematous. Expiration prolonged. Electrocardiogram showed left ventricular preponderance. Roentgenograph: slight increase in linear markings especially in hilar regions. Flaring of the ribs.

Case 3. Male, 55 years. Asthmatic attacks, dyspnea (+++) and cough for 20 years. Chest markedly emphysematous with hyperresonant percussion note, prolonged expiration with scattered musical râles. Marked cyanosis. Electrocardiogram normal. Roentgenograph: increased linear markings.

[*] +   dyspnea only on severe physical activity.
++   dyspnea on moderate exertion.
+++   dyspnea on slight physical activity.
++++ dyspnea at rest.

Case 4. Male, 60 years. No history of asthmatic attacks. Dyspnea (+) and mild cough for 1 year. Emphysematous chest with prolonged expiration. Electrocardiogram presented evidence of myocardial damage. Roentgenograph: increased linear markings, flaring of the ribs.

Case 5. Male, 60 years. Asthmatic attacks for 19 years. Dyspnea (++) and severe cough for 10 years. Chest not emphysematous; hyperresonant percussion note and numerous musical râles. Roentgenograph: marked increase in linear markings, especially at the hilar regions.

Case 6. Male, 63 years. Asthmatic attacks, dyspnea (++) and cough for 40 years. Marked cyanosis and slight clubbing of the fingers. Emphysematous chest with marked hyperresonant percussion note and prolonged expiration; scattered musical râles. Electrocardiogram showed left ventricular preponderance. Roentgenograph: exaggerated linear markings, emphysematous bases.

Case 7. Male, 26 years. Attacks of asthma for 20 years. Dyspnea (+) for 1 year. Negative chest examination. Roentgenograph: increased linear markings and increase in the size of the hilar shadows.

Case 8. Male, 43 years. Asthmatic attacks, dyspnea (++) and cough for 6 years. Chest markedly emphysematous; dorsal kyphosis. Hyperresonant percussion note and prolongation of the expiration. Electrocardiogram normal. Roentgenograph: dense shadow at the right apex. Increased markings.

Case 9. Male, 64 years. Asthmatic attacks, dyspnea (+++) and cough for 30 years. Chest not emphysematous. Percussion note hyperresonant; prolongation of breath sounds with numerous râles. Electrocardiogram showed evidence of myocardial damage. Roentgenograph: increased linear markings, low flat diaphragm, pleural thickening about diaphragm.

Case 10. Male, 52 years. Asthmatic attacks, cough and dyspnea (++) for four and a half years. Chest not emphysematous; percussion note hyperresonant and expiration prolonged. Roentgenograph: increased linear markings and radiability of lung fields, flaring of the ribs, wide intercostal spaces.

Case 11. Male, 52 years. Asthmatic attacks, dyspnea (++) and cough for 5 years. Chest not emphysematous; hyperresonant percussion note, expiration prolonged accompanied by a few scattered musical râles. Roentgenograph: increased linear markings, especially at the hilar zones, increased radiability of the lung fields and flaring of the ribs.

Case 12. Male, 45 years. Asthmatic attacks, dyspnea (+++) and cough for 6 years. Emphysematous chest; hyperresonant percussion note, distant breath sounds with râles and rhonchi. Electrocardiogram showed left ventricular preponderance and sinus tachycardia. Roentgenograph: increased radiability of lung fields, flat diaphragms. Old fibrosis and calcification at right apex.

Case 13. Male, 52 years. Asthmatic attacks, dyspnea (++++) and severe cough for probably 1 year. Emphysematous chest; expiration prolonged *scattered musical râles.* Electrocardiogram showed evidence of myocardial damage. Roentgenograph: prominent linear markings, increase in size and density of hilar shadows. After lipiodol injection a questionable diagnosis of bronchiectasis.

Case 14. Female, 36 years. Asthmatic attacks for 35 years. Dyspnea probably for several years. Emphysematous chest, with hyperresonant percussion note. Electrocardiogram showed sino-auricular tachycardia. Roentgenograph: prominent linear markings with increase in density of hilar shadows; fine feathering over right lung and shadows of increased density in left lung.

Case 15. Male, 32 years. Asthmatic attacks, dyspnea (+) and cough for

12 years. Chest not emphysematous, percussion note hyperresonant, expiration prolonged. Roentgenograph: increased radiability at the lung bases with flaring of the ribs.

Case 16. Male, 41 years. Attacks of asthma, dyspnea (+) and cough for 10 years. Slight clubbing of the fingers. Thorax: hyperresonant percussion note and prolongation of the expiration with few scattered râles. Roentgenograph: increased linear markings, increased radiability of lung fields, flaring of ribs.

Case 17. Male, 41 years. Asthmatic attacks for 10 years. Dyspnea (+++) and severe cough for 26 years. Slight cyanosis of mucous membranes. Emphysematous chest; percussion note hyperresonant, prolongation of breath sounds with numerous musical râles. Electrocardiogram normal. Roentgenograph: increased linear markings especially in both lower fields and in hilar shadows, low flat diaphragm.

Case 18. Male, 38 years. Asthmatic attacks, dyspnea (++) and cough for probably 4 years. Slight cyanosis; marked clubbing of the fingers. Chest not emphysematous; percussion note resonant, prolonged expiration, scattered musical râles. Electrocardiogram showed left ventricular preponderance. Roentgenograph: increased linear markings and enlarged hilar shadows. Dorsal scoliosis.

Case 19. Female, 28 years. Asthmatic attacks, dyspnea (+) and cough for 7 years. Chest normal. Roentgenograph: increased linear markings, especially at the hilar regions.

Case 20. Male, 43 years. Asthmatic attacks, dyspnea (+++) and cough for probably 30 years. Emphysematous chest; prolonged expiration and scattered râles. Electrocardiogram presented left ventricular preponderance. Roentgenograph: increased linear markings, enlarged hilar shadows, low diaphragm, bulging of the ribs. Prominent pulmonary conus.

Case 21. Male, 50 years. Asthmatic attacks, dyspnea (+++) and severe cough for 6 years. Emphysematous chest; hyperresonant percussion note, expiration prolonged. Electrocardiogram normal. Roentgenograph: increased linear markings and enlarged hilar shadows.

Case 22. Male, 17 years. Asthmatic attacks, dyspnea (+) and cough for 1 year. Chest examination negative. Roentgenograph: increased linear markings, more marked at both bases; enlarged hilar shadows.

Case 23. Male, 49 years. Asthmatic attacks, dyspnea (++) and cough for 2 years. Slight cyanosis. Chest not emphysematous, prolonged expiration and scattered musical râles. Electrocardiogram showed left ventricular preponderance. Roentgenograph: increased linear markings with enlarged hilar shadows. Sclerosis of the aorta.

Case 24. Male, 17 years. Asthmatic attack, dyspnea (+) and severe cough for 1 year. Chest normal. Roentgenograph: increased linear markings, especially at the hilar regions.

Case 25. Male, 43 years. Asthmatic attacks, dyspnea (+++) and cough for 5 years. Emphysematous chest; prolonged expiration and scattered musical and coarse râles. Roentgenograph: prominent linear markings with increased radiability at both bases. Pleural thickening.

Case 26. Male, 55 years. No history of asthma. Dyspnea doubtful and cough. Slight cyanosis. Emphysematous chest; hyperresonant percussion note and scattered rhonchi. Electrocardiogram normal. Roentgenograph: slight increase in linear markings with feathering in both lower lung fields; increased radiability of lung fields; calcification of right pleura.

STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS. VII. OBSERVATIONS DURING THE ACUTE RESPIRATORY DISTRESS OF BRONCHIAL ASTHMA AND FOLLOWING THE ADMINISTRATION OF EPINEPHRINE [1]

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In the hope that new light might be thrown on the functional pathology of asthma and the mode of action of epinephrine the total pulmonary capacity and its subdivisions have been measured during the acute stage of bronchial asthma, and the observations repeated soon after the administration of this drug.

METHODS

The methods used in the determination of the total pulmonary capacity and its subdivisions, the nomenclature adopted, and the normal findings, both in males and in females, have been fully presented in previous papers (1), (2). The only modification in the technique previously described is that the determinations were made with the patients in the sitting posture, because some were disturbed by severe orthopnea. In five cases the pulmonary capacity was determined during the acute respiratory distress of the asthmatic attack, and again a few minutes after the subcutaneous administration of epinephrine, when the paroxysm had been relieved. In a sixth case the second determination was made two days later, after the acute symptoms had subsided; in this case no epinephrine had been administered.

CASE ABSTRACTS

A brief clinical summary of the cases studied is presented:

Case 1. Male, aged 42 years. History of hay fever and asthmatic attacks for six and four years respectively. Skin tests indicated sensitiviness to ragweed, feathers, cat dander and dog dander. No dyspnea or cough in the intervals between the attacks. Chest not emphysematous in appearance. Roentgenograph: marked increase in the linear markings, with a flaring of the ribs.

TABLE I

Pulmonary capacity during the acute respiratory distress in bronchial asthma and after its relief by subcutaneous administration of epinephrine
(1 : 1000)

	Case 1		Case 2		Case 3		Case 4		Case 5	
	During attack	After epinephrine	During attack	After epinephrine	During attack	After epinephrine	During attack	After epinephrine	During attack	After epinephrine
cc. of epinephrine injected		0.75		0.75		0.75		0.50		0.75
Minutes after injection		12		10		12		15		20
Total capacity, *liters*	10.02	6.91	5.17	4.82	6.36	6.04	4.74	3.94	5.02	4.11
Vital capacity, *liters*	3.40	4.14	3.54	3.88	2.68	3.06	1.70	2.20	1.60	2.00
Mid capacity, *liters*	8.18	4.39	2.65	2.16	4.30	3.72	3.50	2.04	3.74	2.45
Residual air, *liters*	6.62	2.77	1.63	0.94	3.68	2.98	3.04	1.74	3.42	2.11
Complementary air, *liters*	1.84	2.52	2.52	2.66	2.06	2.32	1.24	1.90	1.28	1.66
Reserve air, *liters*	1.56	1.62	1.02	1.22	0.62	0.74	0.46	0.30	0.32	0.34
Ratio, Vital capacity/Total capacity ×100	33.9	59.9	68.5	80.5	42.2	50.6	35.9	55.9	31.7	48.7
Ratio, Mid capacity/Total capacity ×100	81.6	63.5	51.2	44.8	67.6	61.5	73.8	51.7	74.5	59.6
Ratio, Residual air/Total capacity ×100	66.1	40.1	31.5	19.5	57.8	49.4	64.1	44.1	68.3	51.3
Ratio, Complementary/Total capacity ×100	18.4	36.4	48.7	55.2	32.4	38.4	26.1	48.2	25.5	40.4
Ratio, Reserve/Total capacity ×100	15.6	23.4	19.7	25.3	9.7	12.2	9.7	7.6	6.3	8.2
Ratio, Complementary/Vital capacity ×100	54.1	60.8	71.1	68.5	76.9	75.8	72.8	86.3	80.0	83.0
Ratio, Reserve/Vital capacity × 100	45.9	39.2	28.9	31.5	23.1	24.2	27.2	13.7	20.0	17.0

there was a decrease of the vital capacity together with a marked increase in the mid capacity and residual volume. With the exception of Case 2 these alterations were quite pronounced, and in Case 1 the increase in the residual air was of such magnitude as to give the highest total pulmonary capacity (10.02 liters) which we have yet observed. The relative values (total capacity = 100 per cent) were correspondingly altered. There was a marked decrease of the ratio of vital to total capacity with a proportional
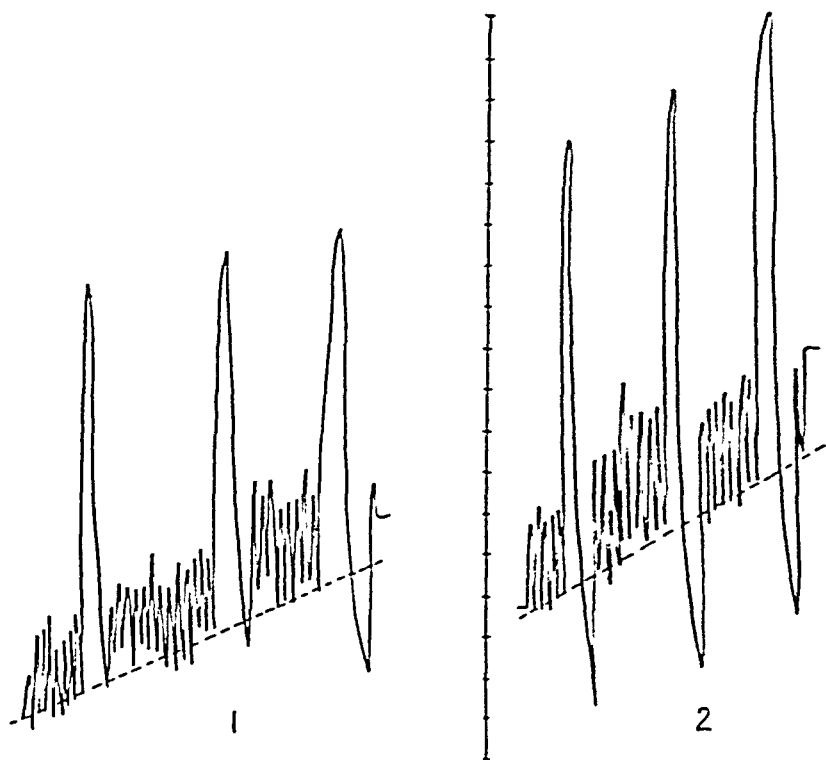
FIG. 2. VITAL CAPACITY TRACINGS FROM CASE 3 DURING THE ACUTE RESPIRATORY DISTRESS OF BRONCHIAL ASTHMA (1) AND SOON AFTER ITS RELIEF FOLLOWING THE SUBCUTANEOUS ADMINISTRATION OF EPINEPHRINE (2).

Notice the disappearance of the reserve air in two of the vital capacity determinations before epinephrine and the increase in the complementary and reserve volumes after epinephrine was administered.

Divisions on the scale correspond to 200 cc. Broken line underlying the tidal breathing is the mid capacity level.

increase in the ratios of the mid capacity and residual air to total capacity. In all cases but one (Case 2), the abnormalities in these ratios were well beyond the normal limits of variation so that they resembled the values observed by us in severe cases of pulmonary emphysema with marked respiratory disability (3). Of the components of vital capacity, the comple-

mentary air appeared most affected, with a consequent relative increase in the reserve volume. We have already mentioned that in all cases the subcutaneous administration of epinephrine produced, after a few minutes, a marked amelioration or disappearance of the respiratory distress experienced by these patients. The changes in pulmonary capacity which accompanied this symptomatic improvement were quite strikingly constant. A definite increase in the vital capacity occurred together with a more marked decrease in the mid capacity and residual air, so that the relative values of these components approximated the normal limits of variation, although never reaching strictly normal values except in Case 2. The total capacity decreased markedly in Case 1 and in a lesser degree in the other patients. The changes which were observed in the vital capacity, and its two components: the complementary and reserve air, as the result of the administration of epinephrine may be appreciated in Figure 2 (Case 3). From Table II it may be seen that when a second injection of epine-

TABLE II

Pulmonary capacity before and after the subcutaneous injection of epinephrine in a case of bronchial asthma with no acute respiratory distress at the time of administration

Pulmonary capacity	Before epinephrine	After epinephrine
c.c. of epinephrine chloride (1%) injected		0.75
Minutes after injection		15
Total capacity, *liters*	7.12	7.11
Vital capacity, *liters*	4.72	4.51
Mid capacity, *liters*	4.48	4.53
Residual air, *liters*	2.40	2.60
Complementary air, *liters*	2.64	2.58
Reserve air, *liters*	2.08	1.93
Ratio, Vital capacity/Total capacity × 100	66.3	63.5
Ratio, Mid capacity/Total capacity × 100	62.9	63.7
Ratio, Residual air/Total capacity ×100	33.7	36.5
Ratio, Complementary/Total capacity ×100	37.1	36.3
Ratio, Reserve/Total capacity ×100	29.2	27.1
Ratio, Complementary/Vital capacity ×100	55.9	57.2
Ratio, Reserve/Vital capacity ×100	44.1	42.8

phrine was given to one of our patients (Case 1) a few days later, during an interval between the asthmatic attacks when no respiratory distress was present, it did not produce any significant alteration in the pulmonary capacity. In Case 6 (Figure 3) in which no epinephrine was given, a comparison of pulmonary capacities determined during the asthmatic attack and two days later when the respiratory distress had subsided reveals the same relative changes as those which occurred in patients to whom epinephrine was given.
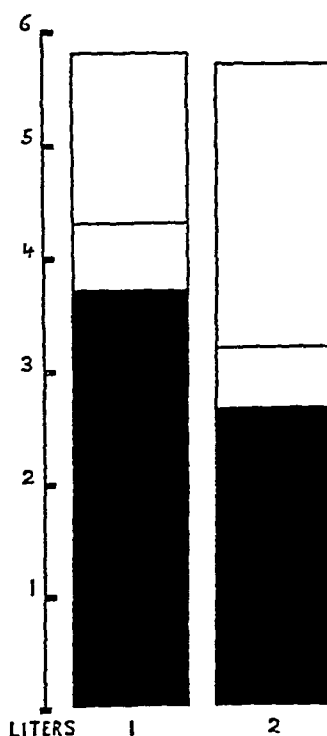
FIG. 3. PULMONARY CAPACITY DURING THE RESPIRATORY DISTRESS OF BRONCHIAL ASTHMA (LEFT COLUMN) AND TWO DAYS LATER WHEN THE ACUTE SYMPTOMS HAD SUBSIDED (RIGHT COLUMN).

No epinephrine was administered in this case.

TABLE III

Pulmonary capacity during and after the acute respiratory distress of bronchial asthma
(No epinephrine administered)

Pulmonary capacity	During attack	After attack
Total capacity, *liters*	5.78	5.72
Vital capacity, *liters*	2.08	3.06
Mid capacity, *liters*	4.30	3.18
Residual air, *liters*	3.70	2.66
Complementary air, *liters*	1.48	2.54
Reserve air, *liters*	0.60	0.52
Ratio, Vital capacity/Total capacity ×100	36.0	53.5
Ratio, Mid capacity/Total capacity ×100	74.3	55.5
Ratio, Residual air/Total capacity ×100	64.0	46.5
Ratio, Complementary/Total capacity ×100	25.5	44.4
Ratio, Reserve/Total capacity ×100	10.3	9.1
Ratio, Complementary/Vital capacity ×100	71.1	83.0
Ratio, Reserve/Vital capacity ×100	28.9	17.0

DISCUSSION

There are few observations in the literature in regard to the determination of the total pulmonary capacity and its subdivisions during the severe respiratory distress of bronchial asthma. We have been unable to find any reference to the changes which occur after the administration of epinephrine in these cases. Means (4) in his monograph on "Dyspnea" refers to investigations made in his own clinic in which an increase of the vital capacity and of the ventilation per minute was observed following the administration of epinephrine in asthmatic attacks. Anthony (5), in 1930, observed changes in pulmonary capacity similar to those mentioned in this communication. Lippelt (6) by narrowing the afferent tube of the Knipping respirator, and thus simulating the bronchial obstruction to expiration as in asthma, produced in healthy subjects an increase in the mid capacity and residual air and a decrease in the complementary volume. Warren (7) in 1913 made orthodiagraphic studies in a case of bronchial asthma and found that the area of the lung fields (measured by means of a planimeter) gradually became smaller as the frequency of the attacks diminished. He also observed that as the patient improved there was an increase in the excursion of the diaphragm. In one of our patients (Case 1), studied fluoroscopically, the maximal excursion of the diaphragm was measured during the asthmatic attack and again after the administration of epinephrine. An increase of almost two centimeters was seen in the second observation.

These observations of the pulmonary capacity during the asthmatic attack aid in understanding the mechanism of the acute respiratory distress experienced by these patients. The marked increase in the residual air and the simultaneous decrease of the vital capacity represent an unfavorable condition for efficient alveolar ventilation. The situation corresponds to that of an extreme case of emphysema, the severe respiratory disability of which has been correlated closely with the degree of change in these two components of the pulmonary capacity. It is also possible that the rapidity with which these changes occur in an asthmatic paroxysm tends to increase the severity of the distress, since there is little time for compensatory adjustments to such an abnormal condition.

Without entering into the evidence as to whether the underlying anatomical and functional mechanism in bronchial asthma is that of bronchiolar edema or bronchiolar spasm or both, it appears to us quite probable that the latter mechanism plays a prominent part. According to Macklin (8) the point of constriction is probably situated in the terminal bronchioles. The narrowing of these structures interferes with expiration, causing a distension of the alveoli and subsequent lessened pulmonary elasticity limiting at the same time the volume of air which may be taken into the lungs in a deep inspiration. Hence the asthmatic patient not only has a

greater amount of alveolar air to be ventilated, but he also suffers from a mechanical limitation of his ability to increase the ventilation per minute. It may be assumed that the action of epinephrine widens the bronchioles, either by relief of their constriction or by diminution of edema of their mucosa, thus permitting rapid and marked decrease in the residual volume, increase in the vital capacity, and consequently more efficient alveolar ventilation.

The consistent character of the changes observed following the administration of epinephrine in these cases of bronchial asthma suggested to us the desirability of studying the effect of acetyl-beta-methylcholin, the broncho-constricting action of which has been demonstrated by Comroe and Starr (9). Starr, Elsom and Reisinger (10), and Starr (11) observed the production of typical asthmatic attacks after the administration of this drug. Our observations are as follows: An intramuscular injection of 30 mgm. was given to a healthy young adult, and three and a half minutes later the vital capacity was found to be 3.12 liters as compared with a previous value of 3.98 liters. In another young normal subject the vital capacity was decreased from 4.10 to 3.40 liters by the administration of 15 mgm. of the drug. The diminution in both of these cases affected chiefly the complementary air. A sense of substernal constriction and difficult breathing were noted in both instances. Attempts to measure the residual air after the administration of this drug were not successful because of the rapidity with which its action disappeared. In both instances, however, a definite lowering of the mid capacity level was noted in the respiratory tracings, about three to four minutes after the onset of the typical effects elicited by the drug (sweating, lowering of the blood pressure, etc.). This change in the tracing probably indicates that an increase in the mid capacity had occurred.

<center>SUMMARY AND CONCLUSIONS</center>

In five cases measurements of the total pulmonary capacity and of its subdivisions were made during the acute respiratory distress of bronchial asthma, and soon after relief of this following the subcutaneous administration of epinephrine. In another case the pulmonary capacity was determined after gradual spontaneous recovery.

Observations were made also of the effect of acetyl-beta-methylcholin on the vital capacity of two normal subjects. These observations led to the following conclusions:

1. During the acute respiratory distress of bronchial asthma there is a decrease in the absolute and relative value of the vital capacity, and a marked increase in the mid capacity and residual air.

2. A few minutes after the administration of epinephrine the vital capacity increases and there is a marked decrease in the mid capacity and

residual air. These changes are accompanied by an amelioration or disappearance of the respiratory distress.

3. The alterations in the pulmonary capacity throw light on the functional pathology of the asthmatic attack, and upon the therapeutic action of epinephrine. Following the administration of acetyl-beta-methylcholin to two healthy subjects, a moderate decrease in vital capacity was observed. This was probably accompanied by an increase in the mid capacity, as indicated by the change in the graphic tracing of the respirations. Accurate measurement of the residual air and mid capacity was impossible on account of the evanescent action of acetyl-beta-methylcholin.

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RADIATION OF HEAT FROM THE HUMAN BODY: A STATEMENT RELATIVE TO THE CRITIQUE OF J. D. HARDY

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Dr. Hardy [1] has made careful recalculations of our experiments on the radiation of the human body. He has found errors in our work and has most kindly called our attention to them. After recalculation I wish to make the following statement:

There is, as a matter of fact, an error which slipped in twice. in that the graphic average value should have been multiplied by the integration-interval. This mistake obviously escaped notice because it was almost compensated by a second error so that the final results came out on the whole approximately correct. We cannot at the present time prove with the old radiometer whether or not these deviations were caused entirely by the difference in wave lengths of the emission of the Hefner lamp used for the calibration and the wave lengths of radiation from the human subjects. The instrument in the meantime has been greatly changed by many alterations in construction necessitated by further experiments. It would be preferable to repeat the entire measurements with a new calibration. In the meantime this has been done by Dr. Hardy. It is therefore of particular importance to us and fortunate for the general development of this field of investigation that the measurements of Dr. Hardy which have avoided our errors lead to the same end results.

In spite of the uncertainty implied by these mistakes we hold fast to our position on the basis of thorough considerations. supported as they are by the results of Dr. Hardy. We believe that our theoretical procedure as well as the method chosen for experimental proof is correct. In this conclusion we have been confirmed by our new investigations of this problem carried on in the last few years.

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[1] Hardy, James D., The radiation of heat from the human body. I. An instrument for measuring the radiation and surface temperature of the skin. J. Clin. Invest., 1934, 13, 593.
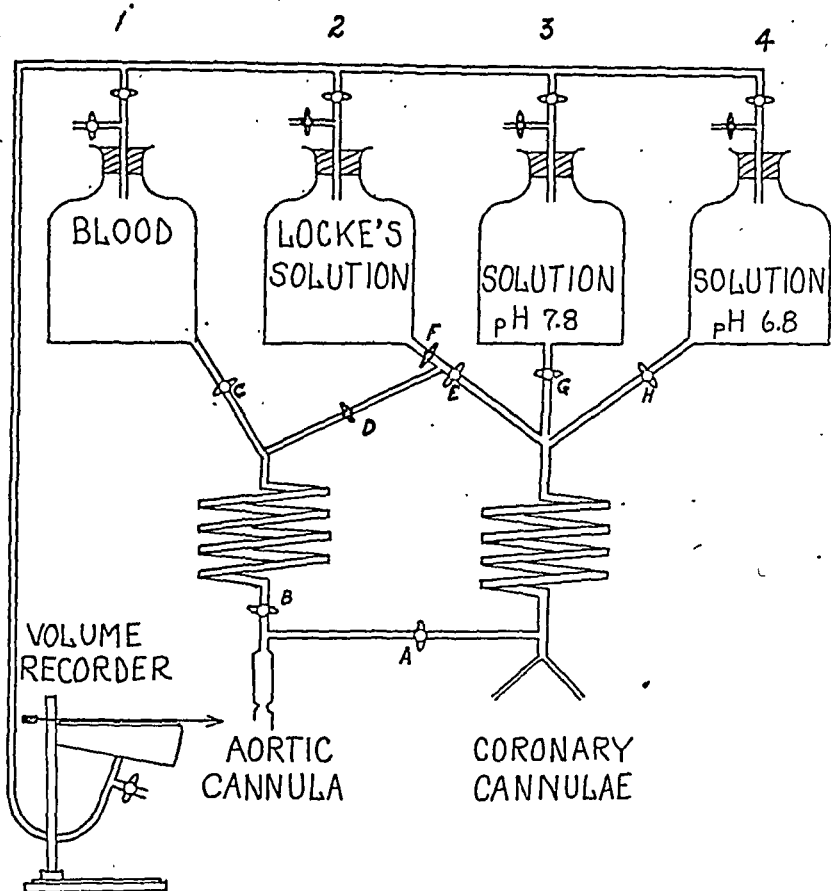
FIG. I. A SCHEMATIC DRAWING OF THE PERFUSION SYSTEM

When it was desired to pass blood into the aorta, stopcocks C and B were opened. By opening stopcock A blood would pass into the coronary cannulae, when C and B were open. To perfuse the aortic cannulae with Locke's solution stopcock C was closed and F, D and B were opened. Opening of E permitted the fluid to pass into the coronary cannulae.

When the heart was arrested with fluid from reservoir 3, stopcocks G and A were opened. After the heart had been stopped, stopcock A was closed, while F, D and B were opened. Thus Locke's solution was perfused into the aorta, while the solution pH 7.8 was perfused into the coronary arteries.

When the heart was arrested with fluid from reservoir 4, stopcocks H and A were opened. After the heart was arrested and the flow into the coronary arteries was being measured, stopcock A was closed and F, D and B were opened.

The level of the fluid in the two reservoirs was kept at the same height above the heart by a screw adjustment attached to the stand, which was carefully adjusted by one observer as the fluid ran out.

at the beginning of the experiment had a pH of 8.0 and at the end a pH of 7.4. Reservoir 4 contained sodium chloride solution of pH 6.6 at the beginning of the experiment and a pH 6.9 at the end. All reservoirs were connected by rubber tubing to the cannulae in the coronary arteries and in the aorta. The outflow from each reservoir could be measured by a volumetric recorder.

Standard rate of coronary flow. The standard rate of flow into the coronary arteries was obtained by lowering the pressure of reservoir 1 and 2 to 80 cm. above the heart (60 mm. Hg). The object of lowering the pressure was twofold. From earlier experiments it was found that perfusion with saline solutions at a high pressure for any considerable period produced edema of the heart muscle, not prevented by addition of acacia to the solution. At a relatively low pressure, however, Locke's solution did not produce edema. The second reason was that the beat of the heart was weak and it has been pointed out by Hausler (2) that under such circumstances, unless the perfusion pressure is low (2), the systolic phase of the heart does not exert its usual effect of inhibiting the coronary flow.

The coronary cannulae were disconnected from reservoir 1 by closing stopcock *A* and opening stopcock *E*. Oxygenated Locke's solution was then passed through the coronary arteries and blood through the aortic cannulae. The rate of flow into the coronary arteries was recorded. In addition, the heart rate and time were registered on a moving drum. After a standard record had been obtained, the vagus and sympathetic nerves were stimulated and the coronary flow and heart rate were again recorded.

Stimulation of nerves in beating heart. It was found that stimulation of the sympathetic nerve as far up as the lower border of the middle cervical ganglion usually gave an accelerator response, therein differing from that of other mammals. The point chosen for stimulation was just below the inferior cervical ganglion. A ligature had been passed around the sympathetic trunk in such a way as to include all branches from the inferior pole of the ganglion and all branches which apparently came from the nerve at this level. The vagus was stimulated at a point just lateral to the middle cervical ganglion, care again being taken to look for and include within the ligature the superior cardiac branch of the vagus nerve when it was present. In three cases it could not be found and presumably was incorporated in the vagus sheath. The nerve was stimulated just peripheral to the ligature which was approximately in the same region in all experiments.

The degree of stimulation was always the same and was determined roughly by the minimum amount of current necessary to produce maximal slowing of the heart when the vagus was stimulated. The secondary coil was usually set at 4 cm. When the vagus had no influence on the ventricular rate the coil was arbitrarily set at 4 cm.

Stimulation of nerves in the arrested heart. After the standard rate of flow had been recorded the pressure was raised to 162 cm. above the body and the heart was perfused from reservoir 1 for a short period. Hearts were then arrested by alkaline or acid solutions according to the method of Iwai (11).

Four hearts were arrested first by perfusion both through the aortic

and coronary cannulae from reservoir 3 which contained a solution at a pH of 8.0, chosen to cause arrest in a state of increased tone (11). The system of reservoirs was adjusted to 80 cm. above the heart (60 mm. of Hg pressure) and fluid from the third reservoir was permitted to flow into the aorta and coronary cannulae by opening stopcock G and A and closing stopcock C and E. No record of the flow was taken during the arrest of the heart. After the heart had stopped the remaining fluid in the reservoir was removed and Locke's solution at a pH of 7.4 was added. This was done because in previous experiments it had been found that no nervous effect could be demonstrated in hearts perfused with a pH so acid or alkaline as to be barely compatible with life. Care was taken in the transfer of fluid to prevent air from entering the perfusion tubes. A record was made of the flow into the coronary arteries of the arrested heart from reservoir 3. The aortic cannula was perfused from reservoir 2 and the flow from this flask was not measured. The vagus and sympathetic nerves were stimulated and a record made.

The heart was then revived by first washing out the vessels with oxygenated Locke's solution followed by blood from reservoir 1 at a pressure of 120 mm. Hg.

In a second series of four individuals the hearts were arrested by perfusion from reservoir 4 containing sodium chloride at pH 6.6, a solution chosen to arrest the heart in a state of decreased tone. The effect of vagus and sympathetic stimulation was observed and the heart was revived as before.

Use of drugs. In order to determine the dependence of the coronary flow on local cardiac vascular factors, the action of five drugs was studied both in beating and in arrested hearts. In these experiments it was desired to compare drugs which have a predominating vascular effect with others which are known to exert their major effect upon the heart muscle.

Histamine, 0.2 cc. 1 : 10,000, was selected as a strong vasodilator (4), and pituitrin, 0.2 cc., for its powerful vasoconstrictor action (5). Epinephrine, 0.5 cc. 1 : 10,000, is known to strengthen the heart beat and to exert its primary effect on the heart muscle, its action on coronary vessels being variable (6) (7). The other substances were CO_2 and large doses of sodium nitrite (5 cc. saturated solution), both of which are thought to dilate the coronary arteries but also to produce dilatation of the beating heart (8).

After the effect of the stimulation of the nerves had been determined in the arrested heart, the drugs were injected into the fluid perfusing the coronary arteries and their effect upon the coronary flow of the heart was noted. The drugs were added after stimulation of the nerves in order to avoid any permanent changes in the capillaries or heart muscle which might influence the effect of the nerves on the coronary flow. The order of injection was: (1) histamine, (2) pituitrin, (3) $NaNO_2$ and (4) epi-

nephrine. Ten minute intervals elapsed between each injection. All of the hearts were revived at the end of the experiment. The action of drugs on the perfused beating human hearts had been previously recorded in 40 hearts other than those in which the nerves had been stimulated (1).

Application of alkaline and acid solutions to coronary vessels. In order to study the effect of acid and alkaline solutions on the coronary vessels, small rings of the coronary arteries were cut and suspended by the method described by Cruickshank and Subba Rau (9). The temperature was raised to 37° C. and acid solution (pH 6.5) and alkaline solution (pH 7.5) were substituted for the Locke's solution in which the rings were suspended. Epinephrine, pituitrin, sodium nitrite and histamine were added to each of the preparations after washing.

<div align="center">RESULTS</div>

Effect of nerve stimulation on the coronary flow of the heart of the beating. In the strongly beating human heart stimulation of the peripheral end of the vagus nerve slowed the heart rate and increased the flow into coronary arteries. Stimulation of the sympathetic nerve increased heart rate and slowed the flow into the coronary arteries.

In four of the hearts there was complete dissociation of the ventricles and auricles. Under these circumstances stimulation of the nerves did not change the ventricular rate but had an exactly opposite effect on coronary flow, vagus stimulation decreasing it while stimulation of the sympathetic increased the flow.

Effect of drugs on the coronary flow of beating hearts Pituitrin was found to slow the heart rate and decrease the coronary flow, showing its vasoconstrictor properties. Histamine increased the flow but did not change to any considerable degree the rate and amplitude of the heart beat demonstrating its vasodilator effect. Sodium nitrite in large doses dilated the heart and increased the coronary flow. CO_2 administered in appreciable amounts weakened the heart beat but increased the flow. The action of epinephrine was variable. Its first effect was an increase in flow which then fell below the standard rate after about 20 seconds (Table I.)

Effect of nerve stimulation on coronary flow in arrested hearts. In hearts stopped by perfusion with a fluid of altered pH the rate of coronary flow was reduced when compared to the beating hearts.

In hearts arrested in a state of increased tone by perfusion with saline solution at a pH of 8.0 to 7.4, vagus stimulation increased the flow into the coronary arteries. Sympathetic stimulation in two cases decreased the coronary flow, while in the other two it had no effect.

When the hearts were arrested in a state of decreased tone by perfusion with saline solution at a pH of 6.6 to 6.9 vagus stimulation had no influence on the coronary flow. Sympathetic stimulation on the other

Effect of drugs on the beating human heart ·

Drug	Heart number	Before drug		After drug	
		Heart rate	Coronary flow*	Heart rate	Coronary flow*
		per minute	*cc. per minute*	*per minute*	*cc. per minute*
Adrenaline 1 cc. 1 : 10,000..........	1	120	155	144	121
	2	86	178	132	138
Pituitrin 1 cc. Park Davis..........	1	118	150	94	90
	2	78	156	62	120
Histamine 2 cc. 1 : 10,000..........	1	123	148	·123	168
	2	72	160	81	168
Sodium nitrite 1 cc. saturated solution.	1	122	153	127	198
	2	84	164	89	212

* Total coronary flow.

hand increased it. In Table II the effect of the stimulation is recorded. The hearts were all viable after completion of the experiments.

Effect of drugs on coronary flow of arrested hearts. In hearts arrested with increased tone (pH 8.0 to 7.4) pituitrin slowed the coronary flow. Histamine, on the other hand, varied in its effect, sometimes increasing and sometimes decreasing the flow. Sodium nitrite in large doses

TABLE II

Coronary flow and heart rate in the beating heart and the coronary flow in the arrested heart

Heart number	Standard flow	Heart rate beats	Flow vagus stimulation	Rate vagus stimulation	Flow sympathetic stimulation	Rate sympathetic stimulation	Standard coronary flow heart arrested by acid	Flow vagus stimulation	Flow sympathetic stimulation	Standard coronary flow heart arrested by alkali	Flow vagus stimulation	Flow vagus stimulation
	cc. per minute	*per minute*	*cc. per minute*	*per minute*	*cc. per minute*	*per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*
1*	75	60	88	44	63	70				35	59	35
2	160	92	173	78	152	102	117	115	135			
3	186	94	195	64	169	112	150	148	166			
4	194	72	210	59	184	89				150	175	108
5†*	65	60	60	64	88	60	55	45	65			
6†	173	65	155	63	191	65				117	142	96
7†	160	72	155	70	180	70				154	176	125
8†*	70	48	57	48	78	52	72	66	93			

* The flow through right coronary artery alone was measured.
† Ventricles beat independently of the auricles.
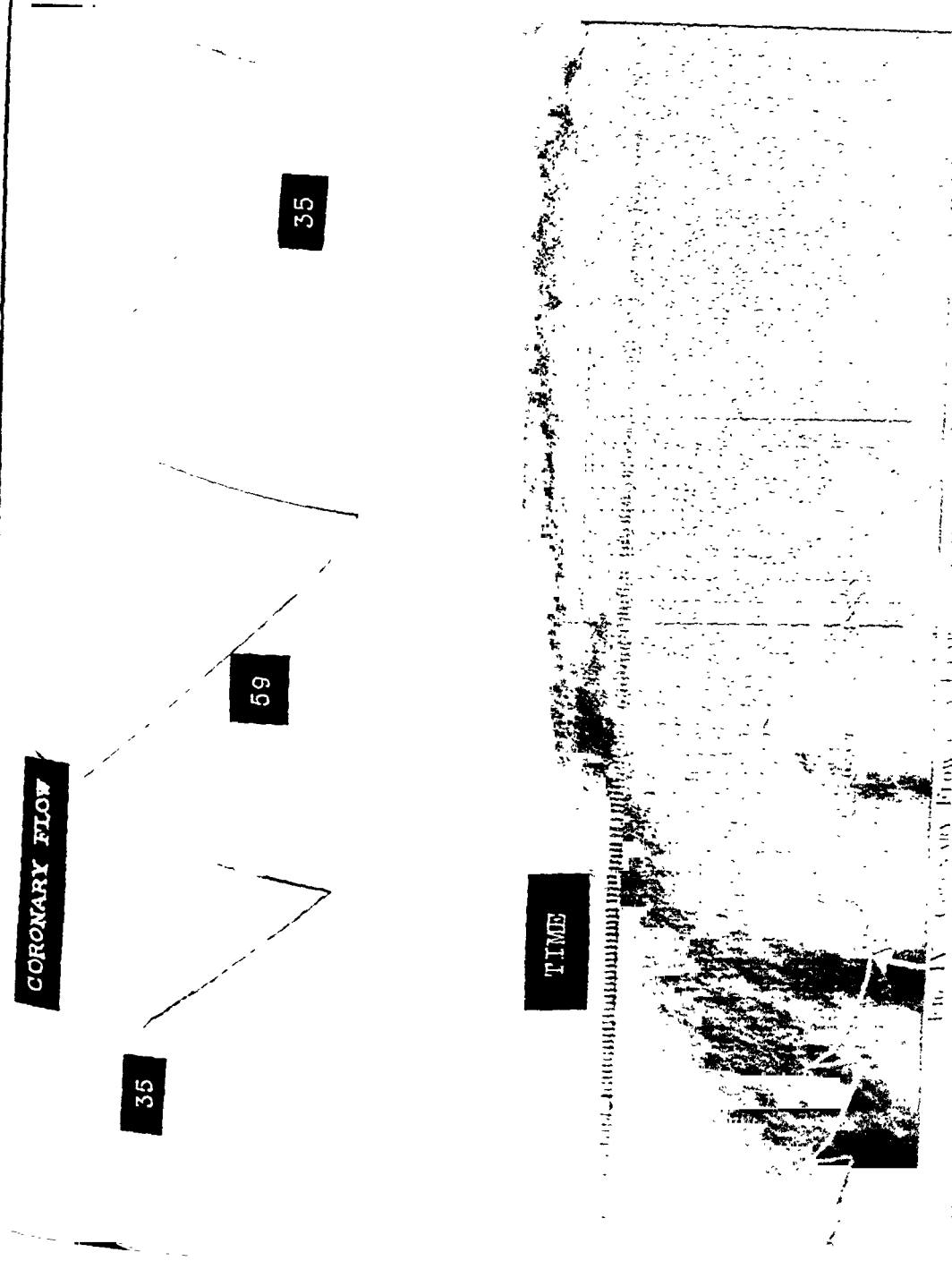
CORONARY FLOW

35   59   35

TIME

in such a heart increased the flow into the coronary arteries. CO_2, in one experiment, likewise increased the flow. Epinephrine definitely slowed it.

In hearts stopped by perfusing with an acid solution (pH 6.6 to 6.9) pituitrin usually slowed the flow into the coronary arteries. Histamine had little effect; sometimes a slight increase was noted when the drug was

TABLE III

Effect of drugs on the coronary flow of arrested human hearts

Heart number	Coronary flow, *cc. per minute*							
	Adrenaline		Pituitrin		Histamine		Sodium nitrite	
	Before drug	After drug	Before drug	After drug	Before drug	After drug	Before drug	After drug
1.* Arrested with alkali..........	40	30	44	34	46	60	48	72
2. Arrested with acid...........	107	116	98	32	99	120	116	125
3. Arrested with alkali..........	143	122	137	115	128	140	137	148
4. Arrested with acid...........	137	152	143	125	140	158	133	142
5.* Arrested with alkali..........	63	57	69	58	70	61	63	76
6. Arrested with acid...........	136	108	123	116	127	150	138	144
7. Arrested with alkali..........	144	127	144	148	134	153	146	168
8.* Arrested with acid...........	60	54	58	54	52	66	58	62

* Flow through right coronary alone measured.

injected into the perfusate. Epinephrine increased the coronary flow while sodium nitrite and CO_2 had no effect.

Effect of drugs on coronary vessels. Immersion of rings of the coronary artery at 37.5° C. into an acid solution resulted in relaxation, while with alkaline solution constriction was observed. Pituitrin added to the alkaline solution usually produced a mild relaxation followed by constriction whereas in vessels relaxed by an acid solution, pituitrin caused constriction. Epinephrine produced a slight constriction in an alkaline solution and in a few experiments some relaxation in an acid solution. Usually no response was obtained by the drug. Sodium nitrite caused a slow prolonged relaxation in an alkaline solution, whereas in an acid solution no effect was noted. Histamine produced constriction in both solutions, usually less in the acid medium.

DISCUSSION

The question may be raised concerning the applicability of our instrument to the solution of this problem. Anrep and Segal (10) employed a string galvanometer so sensitive that it followed coronary flow during each heart beat. Hochrein's and Keller's (13) differential manometer may have been still more accurate. Our volume recorder was much less delicate and could not reveal rapid changes in coronary flow. Since, how-

· TABLE IV

Summary of effects of vagus and sympathetic stimulation and of certain drugs upon the coronary flow

Procedure	State of heart muscle	Changes in coronary flow
Stimulation of vagus nerve	Normal rhythm	Increased
Stimulation of sympathetic nerve	Normal rhythm	Decreased
Stimulation of vagus nerve	A. V. dissociation	Decreased
Stimulation of sympathetic nerve	A. V. dissociation	Increased
Stimulation of vagus nerve	Heart arrested increased tone	Increased
Stimulation of sympathetic nerve	Heart arrested increased tone	Decreased in two experiments
Stimulation of vagus nerve	Heart arrested decreased tone	No change
Stimulation of sympathetic nerve	Heart arrested decreased tone	Increased
Injection of epinephrine	Normal rhythm	Decreased ·
	Heart arrested increased tone	Decreased
	Heart arrested decreased tone	Increased
Injection of pituitrin	Normal rhythm	Decreased
	Heart arrested increased tone	Decreased
	Heart arrested decreased tone	Decreasded
Injection of sodium nitrite	Normal rhythm	Increase
	Heart arrested increased tone	Increased
	Heart arrested decreased tone	No change
Injection of histamine	Normal rhythm	Increased
	Heart arrested increased tone	Variable
	Heart arrested decreased tone	Increased

ever, we were chiefly interested in the change of state of cardiac muscle and since this itself takes place slowly, significant variations in coronary flow should have been apparent.

In a study of the dog's heart Anrep and Segal (10) found that stimulation of the vagus nerve decreased the coronary flow while sympathetic stimulation increased it. We were able to confirm these observations in human hearts when the auricles and ventricles were completely dissociated and the heart rate was not changed by stimulation of the nerves. Confirmation was lacking in hearts with normal mechanism where the rate of the ventricles was influenced by nerve stimulation.

Anrep has attributed his results to a direct effect of the cardiac nerves on coronary vessels, an explanation which may apply to our hearts with auriculoventricular dissociation. With normal cardiac mechanism, however, there was some additional factor which reversed the effect of stimulation of the nerves, and which appeared to be associated with change in the state of the heart muscle at varying rates. A study of Anrep's results, particularly with vagus stimulation, shows a similar phenomenon after a short initial effect in the opposite direction.

Anrep states that rate does not influence the coronary flow in a d. g. This is apparently not true in the human heart unless weakening of the cardiac beat accompanies the changed rate. Thus we have found that a drug like atropine, which increases the rate but has little effect upon the strength of contraction, diminishes coronary flow, while pilocarpine, which

decreases the heart rate without producing changes in the force of the contraction, increases the flow.

The results on arrested hearts can be explained only with difficulty on the basis of direct action of the nerves on coronary vessels. The acceptance of such an explanation would depend upon the assumption of a reversal of nerve effects under the two conditions. While this possibility has been demonstrated by Ten Cate (12), who showed that the action of nerves depends in part upon the ionic concentration of the perfusing solution, there is no evidence that such factors are active under the conditions of our experiments. The theory of changes in cardiac tone offers a more plausible explanation of the results. In the heart arrested by alkalin solutions (increased tone) the vagus nerve might diminish the tone to more nearly normal state and therefore increase the coronary flow. the dilated heart the vagus would lose its effect, but the sympathetic by increasing the tone to a more nearly normal state, might incre flow.[1]

Still further analysis of the phenomenon is possible from th drugs on the arrested hearts and on the rings of the coronary the experiments on coronary rings the drugs which are kn primary vasoconstrictors or vasodilators, if they were active acted the same way, regardless of the solution in which the r pended. On the other hand, those which are known to a the state of the muscle acted differently in different soluti

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SUMMARY

1. The effect of vagus and sympathetic nerve sti flow was studied in the revived human hearts and i rested by alkaline and acid perfusate.

2. In the normal beating human heart vagus s

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[1] The action of the cardiac nerves upon changing the contraction aside from the change in heart rate associate tion has been noted by a number of observers. Chief an have been Gaskell (14), Stefani (15) and Franck-Francoi and Wiggers and Katz (18) have investigated the subjec heart by using systole-cycle ratio to eliminate the effect of to the conclusion that the cardiac nerves, particularly the a a specific effect on the ventricular musculature.

heart rate and increased the coronary flow. Sympathetic nerve stimulation increased the heart rate and slowed the coronary flow.

3. In hearts in which there was dissociation of auricular and ventricular contraction and in which the rate was not influenced by the nerves, vagus stimulation slowed the coronary flow, while sympathetic stimulation increased it.

4. In hearts arrested with increased tone, vagus stimulation increased the coronary flow while sympathetic stimulation in two cases decreased it. In hearts arrested in decreased tone by acid perfusate vagus stimulation had no effect, while sympathetic stimulation increased the flow.

5. The action of the nerves in these hearts was compared to that of drugs. It was found that drugs, which in the beating heart increased muscle action and decreased coronary flow, closely simulated the action of the sympathetic; while drugs, which dilated the beating heart and increased coronary flow, simulated vagus nerve stimulation. No such similarity was noted between nerve action and drugs which act primarily as vasoconstrictors or vasodilators of the coronary vessels themselves.

6. The results of this group of experiments suggest that in man the cardiac nerves exert their most important action on coronary flow through changes in the state of the heart muscle.

The authors wish to express their appreciation of the cooperation of the staff of the Department of Pathology, and especially of Dr. Walter Siebert, for his assistance in some of the experiments.

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8. Bodo, Richard, The effect of the "heart tonics" and other drugs upon the heart tone and coronary circulation. J. Physiol., 1928, 64, 364.

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Still further analysis of the phenomenon is possible from the action of drugs on the arrested hearts and on the rings of the coronary artery. In the experiments on coronary rings the drugs which are known to act as primary vasoconstrictors or vasodilators, if they were active at all, always acted the same way, regardless of the solution in which the rings were suspended. On the other hand, those which are known to act by changing the state of the muscle acted differently in different solutions.

Likewise, in the perfused hearts epinephrine, which increases the force and rate of the heart beat, simulates the action of the sympathetic; while drugs (sodium nitrite and CO_2), which decrease the force of the heart beat, simulate the action of the vagus. On the other hand the action of drugs which have a primary vasoconstrictor (pituitrin) and vasodilator (histamine) effect does not resemble that following stimulation of the nerves.

SUMMARY

1. The effect of vagus and sympathetic nerve stimulation on coronary flow was studied in the revived human hearts and in the same hearts arrested by alkaline and acid perfusate.

2. In the normal beating human heart vagus stimulation slowed the

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[1] The action of the cardiac nerves upon changing the phases of ventricular contraction aside from the change in heart rate associated with nerve stimulation has been noted by a number of observers. Chief among these observers have been Gaskell (14), Stefani (15) and Franck-Francois (16). Katz (17) and Wiggers and Katz (18) have investigated the subject in the mammalian heart by using systole-cycle ratio to eliminate the effect of rate and have come to the conclusion that the cardiac nerves, particularly the accelerators, do have a specific effect on the ventricular musculature.

heart rate and increased the coronary flow. Sympathetic nerve stimulation increased the heart rate and slowed the coronary flow.

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THE PHOSPHATASE CONTENT OF THE BLOOD SERUM IN JAUNDICE [1]

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Enzymes which hydrolyze esters of phosphoric acid. *phosphatases*, are found in many mammalian tissues. Robison (9) has stressed the presence of these enzymes in growing bone and their importance in relation to the processes of calcification and bone development. Further evidence of the importance of this enzyme in the metabolism of bone (6) was adduced with the discovery that the phosphatase content of the blood serum was increased in various diseases such as rickets, Paget's disease or hyperparathyroidism in which osseous changes are marked. In these conditions the increase in the serum or plasma phosphatase apparently is secondary to the osseous lesions.

Phosphatases are present in many tissues and occur in greatest amount in the intestinal mucosa and the kidney. Smaller amounts have been reported in the liver and in the bile (4). Bodansky and Jaffe (2, 3) have found that in normal individuals the phosphatase content of the serum varies with digestive activity and so postulate that while some of the phosphatase in the serum may be osseous in origin some at least is non-osseous. This is also true in some pathological conditions for Roberts (7, 8) who was one of the first to study the changes in the phosphatase content of the serum in disease, reported that high values were present in cases of obstructive jaundice whereas in catarrhal jaundice the value was only slightly increased over the normal. Roberts (8) considered this difference to be sufficiently marked to be of diagnostic value in the differentiation of the several types of jaundice. Bodansky and Jaffe (2) confirmed the increase in the serum phosphatase in jaundice but their results cast doubt on the diagnostic value of the test for they reported a series of nine cases of catarrhal jaundice and hepatitis in which elevated readings were obtained.

METHODS AND MATERIALS

Using the method of Bodansky (1) we have studied the phosphatase content of the serum in a series of 40 cases of jaundice of various types,

TABLE II

The phosphatase content of the serum in obstructive jaundice

Case number	Age	Sex	Date	Serum phosphatase	Icterus index	Serum bilirubin	Van den Bergh reaction	Diagnosis
	years			units per 100 cc.		mgm. per 100 cc.		
9	62	F.	November 22, 1933	57.7	44	7.2	+	Common duct stone
			November 29, 1933	46.4	47	6.0	+	Obstructive cirrhosis
			January 17, 1934	39.2	23	2.3	+	
			January 31, 1934	36.1	33	2.2	+	
			February 28, 1934	47.9	136	17.9	+	
			March 7, 1934	53.0	120	16.0	+	
10	65	M.	March 18, 1933	64.5	37	2.8	+	Chronic cholecystitis with stones
			April 7, 1933	35.3	15	1.0	+	Cholangitis. Cholecystectomy March 12, 1933
11	25	M.	February 14, 1933	42.0	23	2.8	+	Common duct stone
			February 16, 1933	26.4		Less than 2	0	
12	60	F.	April 12, 1933	31.6	120	19.5	+	Chronic cholecystitis with stones
			April 24, 1933	19.3	75	4.0	+	
			May 22, 1933	20.8	250	15.8	+	Following colic
			May 23, 1933	18.6	40	9.0	+	After duodenal drainage
13	48	M.	November 5, 1933	21.7	125	15.7	+	Chronic cholecystitis with stones
			November 10, 1933	14.0	71	5.5	+	Cholecystectomy November 3, 1933
14	70	F.	October 10, 1933	17.5	100	11.3	+	Chronic cholecystitis with stones
			November 2, 1933	11.6	21	2.0	+	Cholecystectomy October 9, 1933
15	46	M.	October 17, 1933	12.6	108	10.7	+	Common duct obstruction—stone
			October 23, 1933	14.0	65	6.0	+	Acute subsiding cholecystitis
			November 20, 1933	13.4	20	2.0	+	Choledochostomy. Cholecystectomy
16	50	M.	October 31, 1933	12.7	40	5.8	+	Chronic cholecystitis. Postoperative biliary fistula
17	49	F.	December 21, 1932	20.6	35	4.5	+	Common duct stone
18	59	M.	December 14, 1933	22.3	125	15.7	+	Postoperative stricture
			December 18, 1933	21.0	150	20.8	+	Common duct
			December 21, 1933	14.1	158	21.1	+	Operation December 18, 1933
			December 26, 1933	14.6	100	15.3	+	

TABLE III

The phosphatase content of the serum in hepatic jaundice

Case number	Age	Sex	Date	Serum phosphatase	Icterus index	Serum bilirubin	Van den Bergh reaction	Diagnosis
	years			*units per 100 cc.*		*mgm. per 100 cc.*		
19	27	M.	February 23, 1934	44.8	300	46.8	+	Acute hepatitis
			February 25, 1934	72.2	375	47.3	+	
			March 2, 1934	35.6	273	42.4	+	
			March 6, 1934	52.2	204	25.0	+	
			March 10, 1934	68.4	167	14.0	+	
			March 28, 1934	53.9	136	12.2	+	
20	26	M.	March 7, 1933	54.5	166	17.3	+	Acute hepatitis
			March 22, 1933	14.5	64	7.8	+	
			April 7, 1933	50.7	38	2.5	+	
21	34	M.	April 24, 1933	20.4	55	18.0	+	Acute hepatitis
			May 3, 1933	8.2	21	3.7	+	
			May 24, 1933	8.6	13	3.2	+	
22	38	M.	September 16, 1933	10.4	125	11.7	+	Subacute hepatitis
			October 2, 1933	10.2	125	22.5	+	
			October 12, 1933	11.5	100	13.1	+	
			October 17, 1933	8.4	75	7.4	+	
23	10	M.	May 4, 1933	28.8	200	5.0	+	Acute hepatitis
24	51	M.	April 25, 1932	12.4		10.7	+	Acute hepatitis
25	27	M.	December 26, 1933	10.6	38	3.1	+	Acute hepatitis
26	29	F.	April 25, 1932	9.5		6.8	+	Acute hepatitis
27	42	M.	March 27, 1933	18.4	105	7.3	+	Subacute hepatitis, syphilitic
			March 31, 1933	13.9	150	6.8	+	
			April 7, 1933	12.1	86	5.4	+	
			April 19, 1933	13.3	47	3.4	+	
			April 27, 1933	11.7	30	2.7	+	
28	48	M.	April 19, 1933	20.6	176	17.4	+	Acute hepatitis, syphilitic
			May 12, 1933	12.2	150	18.8	+	
			May 18, 1933	12.4	100	10.7	+	
			May 24, 1933	11.8	30	4.0	+	
29	63	M.	May 15, 1933	10.4	37	5.0	+	Syphilis, post-arsphenamine jaundice
30	22	F.	October 12, 1933	58.1	71	4.4	+	Acute septic hepatitis

ever, there was no definite correlation between the changes in the phosphatase and the bilirubin content of the serum.

Eight cases of hepatic cirrhosis were studied (Table IV) and the phosphatase was elevated in five. Normal readings were obtained in two cases of congenital hemolytic jaundice.

TABLE IV

The phosphatase content of the serum in hepatic cirrhosis and hemolytic jaundice

Case number	Age	Sex	Date		Serum phosphatase	Icterus index	Serum bilirubin	Van den Bergh reaction	Diagnosis
	years				units per 100 cc.		mgm. per 100 cc.		
31	37	M.	April	26, 1933	11.7	7	1.4	0	Portal cirrhosis. Ascites
			February	27, 1934	12.4	136	17.5	+	Toxic hepatitis with
			March	5, 1934	8.2	107	14.6	+	jaundice
			March	6, 1934	13.5	115	14.2	+	
			March	10, 1934	15.7	97	7.9	+	
			March	14, 1934	13.9	136 (hem.)	11.3	+	
32	33	M.	February	25, 1933	1.3	122	30.5	+	Portal cirrhosis—No ascites. Toxic hepatitis with jaundice
33	46	M.	December	28, 1933	21.8	19	3.0	+	Portal cirrhosis of the liver due to CCl_4 poisoning. Ascites
34	57	M.	May	22, 1933	19.9	5		0	Portal cirrhosis
35	40	M.	July	19, 1933	11.8	17	3.5	+	Portal cirrhosis. Ascites
36	59	M.	October	18, 1933	15.4	11	2.0	0	Portal cirrhosis. Ascites
37	29	M.	January	31, 1933	8.6	15	2.0	+	Portal cirrhosis. No ascites. Wilson's disease
38	68	M.	May	16, 1934	9.2	14	1.3	±	Obstructive biliary cirrhosis
39	14	F.	April	20, 1934	9.9	167	24.4	0	Congenital hemolytic jaundice
40	24	M.	June	13, 1933	3.2	88	5.8	0	Congenital hemolytic jaundice

The phosphatase was determined in bile obtained by duodenal drainage in a series of 15 cases of chronic cholecystitis without jaundice of one type or another (Table V). Phosphatase was present in all, but the amount varied widely, the lowest reading being 5 units per 100 cc. and the highest

224.   In those cases in which the duodenal and concentrated specimens were both studied the activity was increased in the latter.   This would suggest that the phosphatase activity was increased with the concentration of bile in the gallbladder.   The phosphatase activity was also determined in a few specimens of bile removed from the gallbladder at operation. These readings are not included in the table but were similar in magnitude to those reported there.   Case 16 in Table V shows the phosphatase con-

TABLE V

The phosphatase content of bile obtained by duodenal intubation

Case number	Age	Sex	Date	Phosphatase		Bile acids	
				Duodenal bile	Concentrated bile	Duodenal bile	Concentrated bile
	years			units per 100 cc.	units per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
1	27	F.	November 22, 1933		8		465
2	32	F.	November 15, 1932	0	5	180	484
3	24	F.	November 15, 1932	44	224	240	265
4	47	F.	January 18, 1933	5	102	74	182
5	43	F.	January 8, 1933	51	61	99	93
6	42	F.	November 15, 1933	25	97	80	91
7	32	F.	November 18, 1933	5	199	82	488
8	56	F.	October 25, 1933	51	53	0	484
9	32	F.	October 25, 1933	69	86	143	545
10	24	F.	December 6, 1933	5	130	0	408
11	55	F.	November 29, 1933	13	85	0	164
12	36	F.	November 19, 1933	0	5	23	438
13	34	F.	November 22, 1933	22	28	50	188
14	62	F.	November 22, 1933	28	30	59	96
15	35	M.	November 29, 1933	16	29	83	182
16	55	F.	July 27, 1933	59		335	

tent of bile obtained by surgical drainage of the gallbladder in a patient with acute pancreatitis and total destruction of the pancreas.

DISCUSSION

The finding of phosphatase in bile obtained by duodenal drainage is not conclusive evidence for the hepatic origin of the enzyme.   Bile obtained from the gallbladder at operation frequently contains amylase and it is usually assumed that the presence of amylase indicates the back flow of a small amount of pancreatic juice into the gallbladder.   We have found phosphatase in bile obtained from the gallbladder at operation and this too may be extra-hepatic in origin.   On the other hand the constant finding of phosphatase in specimens of bile, whether obtained at operation or by duodenal drainage, and the greater concentration in specimens from the gallbladder speaks for the hepatic origin of a considerable part of the en-

zyme. Certainly the constancy of these observations would be difficult to explain on the assumption that the whole of the phosphatase came from pancreatic or duodenal juice which was present as a contaminant. The complete destruction of the pancreas in the case cited would further serve to exclude this organ as the source of the phosphatase found in the bile.

The present results confirm the earlier studies of Roberts (8) in demonstrating an increase in the phosphatase content of the serum in obstructive jaundice. This increase is further evidence for the hepatic origin of the phosphatase. The phosphatase content of the serum, however, contrary to the findings of Roberts (8) and in accord with those of Bodansky and Jaffe (2), is increased in cases of hepatitis as well as in obstructive jaundice. In our experience the test has been valueless in the differential diagnosis of the two types of jaundice. Hartman (5) has found that the serum phosphatase is increased in animals with experimentally produced cirrhosis of the liver. These observations are in agreement with our findings of an increase in the serum phosphatase in cases of hepatic cirrhosis without jaundice. These findings, together with the lack of correlation between the increase in the phosphatase and the elevation of the serum bilirubin, further suggest that in cirrhosis the serum phosphatase is a measure of hepatic disturbance rather than a consequence of the jaundice per se.

The finding of normal values in the two cases of congenital hemolytic icterus further emphasizes both the independence between the phosphatase and bilirubin and the difference in the pathogenesis of hemolytic icterus and of the obstructive and hepatic types of jaundice.

SUMMARY

Phosphatase was present in samples of bile obtained from the gallbladder at operation or by duodenal intubation.

The phosphatase content of the serum was increased in cases of jaundice due to hepatitis or to obstruction of the biliary passages. This test was of no value in the differential diagnosis of these two conditions. The phosphatase content of the serum was not increased in cases of hemolytic jaundice.

The phosphatase content of the serum was increased in cases of portal cirrhosis.

These findings suggest that the phosphatase in the bile probably is hepatic in origin and that some of the phosphatase normally present in serum is non-osseous and possibly hepatic in origin.

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